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July 30, 1948

Science

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(See Page 99)

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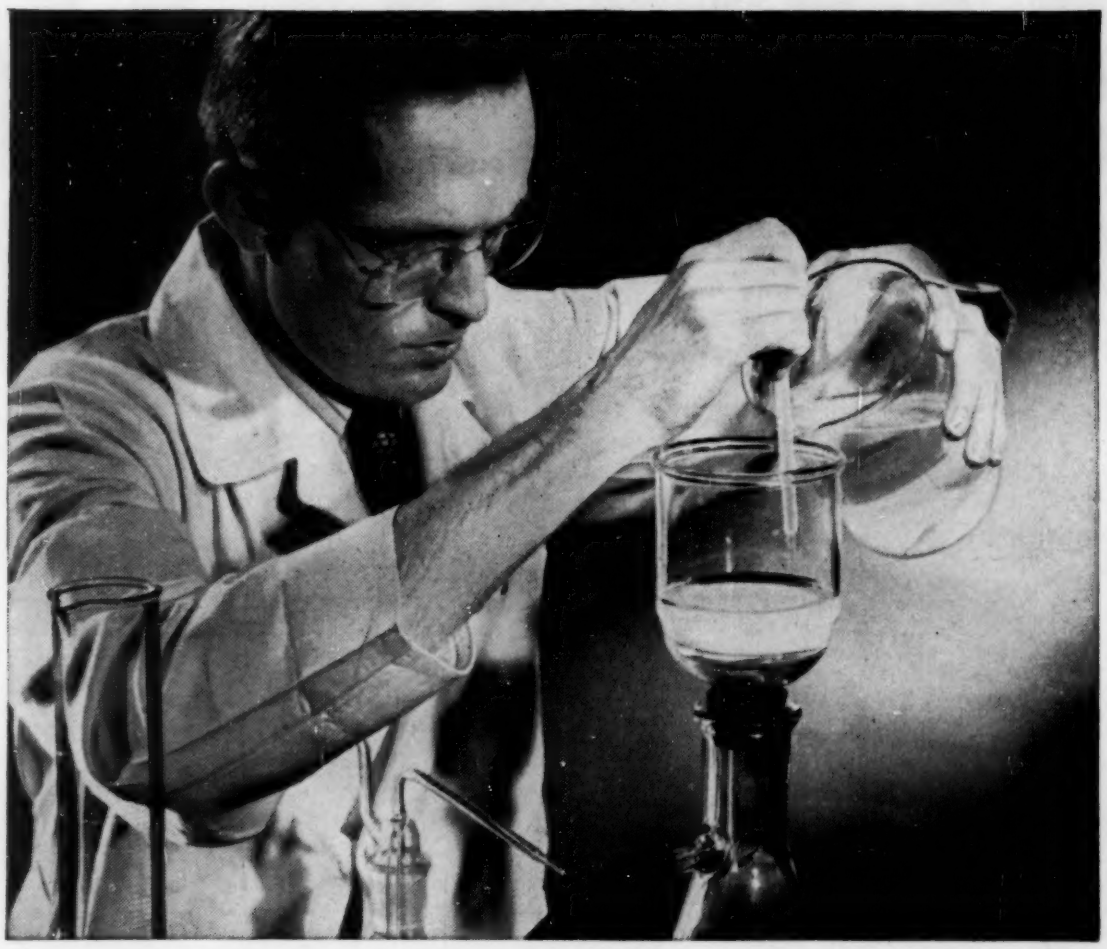

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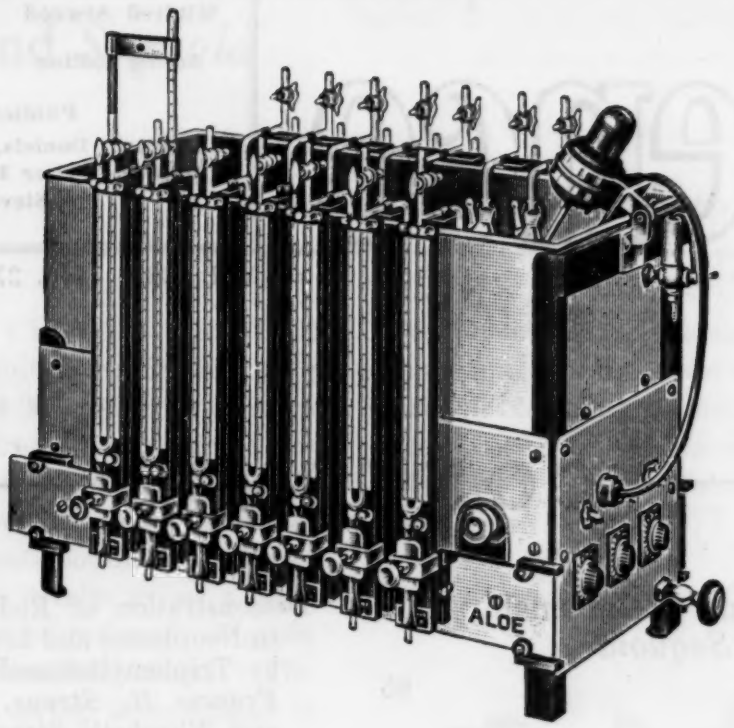

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CONTENTS

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The Chromosomes and Relationships of *Metasequoia* and *Sequoia*

G. L. Stebbins, Jr.
University of California, Berkeley

THE DISCOVERY BY CERTAIN CHINESE foresters, and the recognition by Profs. Cheng and Hu, of living trees belonging to a genus described a few years earlier from fossil material was related in an earlier issue of this journal by Merrill (*7*). To this genus, *Metasequoia,* belong the great majority of the fossils which paleobotanists had previously judged to be the same species as, or a close relative of, the California coast redwood, *Sequoia sempervirens* (Chaney, oral communication). The systematic description of the living *Metasequoia* is either unpublished or, if published, has not yet been available to the present writer, although he has seen a copy of the illustration which Profs. Hu and Cheng are including with their description. Nevertheless, the extraordinary interest of this plant and the fact that both living and preserved material of it are now available make possible and desirable at least a preliminary evaluation of its relationships. The discussion and opinions presented here are based on examination of a series of herbarium specimens collected both in 1946 and by the 1947 expedition sponsored by the Arnold Arboretum and described by Dr. Merrill; of seedlings grown by N. T. Mirov, of the California Forest and Range Experiment Station, from seeds collected by the 1947 expedition; and of young trees as well as of microsporangiate and ovulate strobili in the stage of pollen shedding and pollination, collected by Ralph W. Chaney in March 1948.

A chromosome count of *Metasequoia* was obtained from acetocarmine smears of a vegetative shoot taken from one of the young trees brought back by Dr. Chaney and fixed in a 4:3:1 mixture of chloroform, absolute alcohol, and glacial acetic acid after a pretreatment of one hour in an 0.2% aqueous solution of colchicine. The chromosome number is 2n = 22, and the chromosomes are similar in both size and morphology to those illustrated by Jensen and Levan (*6*) for *Sequoiadendron giganteum* (Fig. 1). Other counts reported for the family Taxodiaceae are $n = 11$ in *Cryptomeria japonica,* 2n = "about 22" in *Taiwania cryptomerioides* and *Taxodium distichum* (*8*), and 2n = 20 in *Sciadopitys verticillata* (*10*). The writer has verified the number 22 in shoot smears of a tree of *Taxodium distichum* growing on the University of California campus and has found the chromosomes to be essentially similar to those of *Sequoiadendron* and *Metasequoia.*

For *S. sempervirens* the counts recorded by various workers are n = 16, 2n = "about 50," and n = 22 (*2, 6*).

FIG. 1. Somatic chromosomes of *Metasequoia* from an acetocarmine preparation of a shoot smear (×1,900).

Seven years ago the writer attempted to study meiosis from acetocarmine preparations of microsporogenesis made from trees of *S. sempervirens* growing on the University of California campus, but in nearly all of the sporocytes the chromosomes were so closely packed together that they could not be separated well enough for counting without breaking individual chromosomes. Nevertheless, one or two cells could, with a little interpretation, be analyzed approximately in their entirety, and the chromosome complement of such a cell at first metaphase is illustrated in Fig. 2.

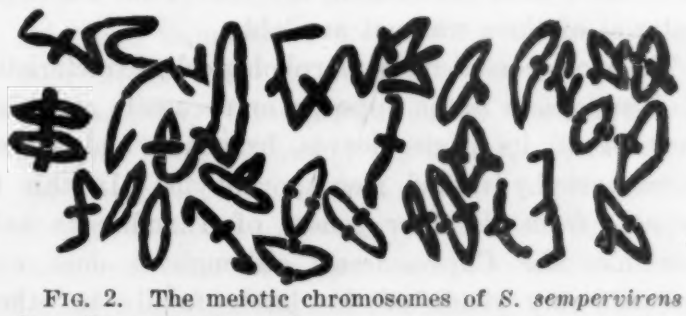

FIG. 2. The meiotic chromosomes of *S. sempervirens* from first metaphase of microsporogenesis acetocarmine smear (×1,400).

It shows 2 chains of 6, 2 quadrivalents, and 22 bivalents, adding up to 2n = 66 chromosomes. In another cell the complement was approximately 3

chains or rings of 6, 3 quadrivalents, and 18 bivalents. In two different chromosome groups at first anaphase, a haploid number of about 33 was counted. Finally, somatic smears of both leafy shoots and ovules, some of which were made during the current year, yielded a few counts of approximately 66 and several of more than 50. Some of these made from the same tree, growing on the campus of Stanford University, from which Dr. Buchholz obtained the material which was the basis of his count of n = 22 (2n = 44). Furthermore, in all of these preparations of somatic tissues, 6 nucleoli were clearly seen in many telophase or resting nuclei. The writer is reasonably certain, therefore, that the coast redwood is a hexaploid with a somatic number of about 66 chromosomes. No other natural polyploids are known in the Taxodiaceae, and only a few have been recorded for the entire order Coniferales (*6*).

The cytological evidence, therefore, tells us little about the interrelationships between the genera of Taxodiaceae, except that *S. sempervirens* must have been derived from one or more other species not very different from it genetically and morphologically by means of polyploidy and perhaps hybridization. This intriguing problem will be discussed below, after a discussion of the morphological characteristics of the genera which appear to be most closely related to *Sequoia* and *Metasequoia.*

Merrill (*7*) has said of *Metasequoia* that "its botanical alliance is scarcely with *Sequoia,* as one might infer from its generic name. In its vegetative characters it suggests *Glyptostrobus* and *Taxodium,* but it may prove to be not closely allied to either of these two genera. . . ." This indicates that the four genera which should be considered as most closely related to *Metasequoia* are *Glyptostrobus,* the Asiatic "water pine"; *Taxodium,* the bald cypress of the eastern United States and Mexico; *S. sempervirens,* the redwood of the California coast; and *Sequoiadendron giganteum,* the "big tree" of the Californian Sierra Nevada. Two other genera of Taxodiaceae, *Taiwania* and *Athrotaxis,* may be as closely related as some of those included in this study, but adequate material of them was not available.

The most conspicuous morphological characteristic of *Metasequoia* is the opposite or decussate arrangement of all its parts: leaves, branches, bud scales, microsporophylls, and megasporophylls. In this it departs from all other genera of Taxodiaceae and resembles the Cupressaceae, although it does not resemble any genus of the latter family in other characteristics. The next most striking vegetative characteristic, the deciduous nature of the foliage and branchlets, is shared with *Taxodium* and *Glyptostrobus.* However, there is some evidence that this resemblance is due to parallel evolution rather than to true relationship. The cataphylls or winter bud scales of *Metasequoia* are much larger and more numerous than those of *Glyptostrobus* and *Taxodium,* and their epidermal cells are very different in appearance. In this connection the fact must be noted that *Sequoia sempervirens,* although evergreen, forms winter buds covered by cataphyll-like scales of about the same size and shape as those of *Metasequoia,* although in the California redwood these structures are thicker, usually green, and bear stomata on their outer (abaxial) surface. The branchlets of *Sequoia* disarticulate at the position of these scales, just as they do in *Metasequoia.* In *Sequoiadendron* the leaves are all similar, and no structures resembling bud scales are found.

In the morphology of its leaves, *Metasequoia* differs from all four of the other genera in that only flat, needle-like leaves are found. The leaves of *Sequoiadendron* are all scale-like or acicular; those of *Sequoia* and *Taxodium* are either needle-like or scale-like; while *Glyptostrobus* possesses scale-like, acicular, and needle-like leaves, with all three types often occurring on the same tree (*5*). A cursory microscopic examination of the leaf epidermis of *Metasequoia* indicates that the orientation and cellular structure of the stomatal apparatus resemble those of *Sequoia, Sequoiadendron,* and *Glyptostrobus,* as described by Florin (*4*) and observed also by the present writer. *Taxodium* differs in the irregular, usually transverse orientation of its stomata. There are, however, certain anomalous features of the leaf epidermis of *Metasequoia* which require further study.

Metasequoia resembles *Taxodium* in the arrangement of its pollen-bearing cones racemosely on specialized branches. When shedding pollen, the individual cones are short-stiped, as in *Sequoia, Taxodium,* and *Glyptostrobus,* while in *Sequoiadendron* they are sessile (*3*). The decussate arrangement of the microsporophylls or cone scales is unique among the Taxodiaceae. They bear on the summit of a conspicuous stalk an ovate sterile tip and three microsporangia, being in all of these respects very similar to the pollen-bearing scales of *Sequoia* and *Sequoiadendron.* Those of *Glyptostrobus* and *Taxodium* have much shorter stalks, and more numerous (6–9) smaller microsporangia (*5*).

The ovulate cones are also racemosely arranged along the branches, emerging in spring from lateral buds covered with numerous conspicuous cataphylls and toward maturity becoming elevated on elongate naked or scaly peduncles. In contrast, the ovulate cones of all other genera of Taxodiaceae are formed on the ends of twigs formed in the previous season or seasons. The decussately arranged sporophylls, when young, are similar to those of *S. sempervirens,*

bearing about 8 ovules in a single row. In *Sequoiadendron* the ovules are arranged in two rows (*2*), while in both *Glyptostrobus* and *Taxodium* the much narrower sporophylls bear only two ovules. In *Metasequoia,* as in *Sequoia* and *Sequoiadendron* (as well as *Athrotaxis, Taiwania,* and *Cunninghamia*), the growth of the cone scale during maturation produces an inversion of the position of the seeds, so that at maturity they are reflexed, with their micropylar or apical ends pointing toward the axis of the cone. In *Glyptostrobus* and *Taxodium,* on the other hand, they remain erect until maturity. The seeds of *Metasequoia* resemble closely those of *Sequoiadendron,* having two conspicuous, pale wings on either side of a narrow body. The seeds of *Sequoia* have somewhat narrower, darker-colored wings, but are otherwise similar. Those of *Glyptostrobus* have no lateral wings, but a wing-like structure at their base, while the seeds of *Taxodium* are angular, thick, and wingless. The scales of the mature cones are essentially similar in *Metasequoia, Sequoia,* and *Sequoiadendron,* except that in the latter genus they are much larger. Those of *Taxodium* are somewhat similar, but *Glyptostrobus* has much narrower, oblanceolate cone scales. Judging from the considerable number of seedlings raised by Dr. Mirov, the number of cotyledons in the embryo of *Metasequoia* is consistently two, which is the usual number in *S. sempervirens*. *Sequoiadendron* ordinarily has four cotyledons, while the usual number in both *Glyptostrobus* and *Taxodium* is six.

These comparisons can be summed up as follows: In the decussate arrangement of its leaves and sporophylls, as well as in the character of its bud scales or cataphylls, *Metasequoia* is unique among the Taxodiaceae. Its leaves, although deciduous like those of *Glyptostrobus* and *Taxodium,* are perhaps more similar in structure to those of *Sequoia* than to those of any other genus in the family. In arrangement of the microsporangiate or pollenbearing cones, *Metasequoia* resembles *Glyptostrobus* and *Taxodium,* but the cones themselves, particularly with respect to the structure of the cone scales or sporophylls, are most like *Sequoia*. The racemose arrangement of the ovulate cones is likewise unique, but the cone scales, ovules, seeds, and young seedlings are much more like those of *Sequoia* and (in the case of the seeds) *Sequoiadendron* than those of *Glyptostrobus* and *Taxodium*.

Summing up the resemblances and differences in respect to 27 characters, including all those on which data could be obtained, and applying a simplification of Anderson and Abbe's (*1*) method of aggregate differences, the following conclusions were reached: *Metasequoia* resembles *Sequoia* in the largest number of these characters (18), differing from it in only 9. The next closest genus is *Taxodium,* with 14 similarities and 13 differences; then comes *Glyptostrobus,* with 12 and 15; and finally, *Sequoiadendron,* with 11 similarities and 16 differences with respect to *Metasequoia*. If spheres representing the five genera are joined by lines whose length is proportional to the number of differences separating each pair of genera, a three-dimensional figure of irregular shape is produced. This cannot be reproduced accurately on a sheet of paper, but its shape approaches that of a fusiform polygon with *Taxodium* and *Glyptostrobus* rather close together at one end, *Sequoiadendron* isolated at the other, and *Sequoia* and *Metasequoia* somewhere in the middle. The nearest genus to *Sequoiadendron* is definitely *Sequoia,* but the number of differences between these two ($10\frac{1}{2}$) is larger than that between *Sequoia* and *Metasequoia* (9). Since the striking differences between the latter two genera which have been mentioned above would preclude the possibility of uniting them into a single genus, the presence of *Metasequoia* strengthens the point of view adopted by Buchholz (*2, 3*) in recognizing the generic distinctness of *Sequoia* and *Sequoiadendron*. If the distinguishing characters are all considered to be of equal importance, *Sequoia* must be regarded as closer to *Metasequoia* than to *Sequoiadendron*. Furthermore, the only type of emphasis of characters which would lead to the opposite viewpoint would be one which considered certain vegetative characters (arrangement of leaves, deciduous vs. evergreen character) more important than differences in the structure of the reproductive organs (sessile vs. stipitate microsporangiate strobili, number of ovules per megasporophyll, length of time for maturation of seeds, shape of cone scales, number of cotyledons). In most groups of seed plants, these reproductive characters are considered more important than the vegetative ones. In regard to *Glyptostrobus* and *Taxodium,* the writer's opinion is that they are not related to *Metasequoia* more closely than is indicated by the placing of all of these genera in the family Taxodiaceae. The deciduous character of the leaves and the racemose arrangement of the cones, both of them clearly derived characters, have probably been acquired by *Metasequoia* independently of their appearance in *Glyptostrobus* and *Taxodium*. This opinion, of course, is subject to change as new evidence from additional characters is obtained. Of particular value would be a study of the development of gametophytes and embryos in *Metasequoia,* since these structures have been made classic botanical material by the researches of Chamberlain, Saxton, Looby and Doyle, Buchholz, and others.

Finally, the question of the origin of polyploidy in *S. sempervirens* should be considered. The configuration of the chromosomes at the meiotic metaphase in

this species (Fig. 2) suggests that it is neither an autopolyploid or a typical allopolyploid, but one of the two intermediate types discussed elsewhere (*9*). It is either an autoallopolyploid with the genomic formula AAAABB, or a segmental allopolyploid with either $A_1A_1A_1A_1A_2A_2$ or $A_1A_1A_2A_2A_3A_3$. In any case, the trebling of the chromosome number was probably accompanied by hybridization between at least two, and perhaps three, distinct ancestral species. The comparison of characters summarized above has suggested to the writer that *S. sempervirens* could not have resulted from combining the characteristics of any two of the four diploid types mentioned and the other existing genera of the Taxodiaceae; *Cunninghamia, Taiwania, Cryptomeria,* and *Athrotaxis* do not seem to possess the characters found in *S. sempervirens* which are lacking in *Metasequoia, Sequoiadendron, Glyptostrobus,* and *Taxodium.* There is good reason to believe, therefore, that at least one of the diploid ancestors of this polyploid is extinct and has left no close relatives. If, however, one of these diploid ancestors is surviving or has left a close living descendant, this species would have to be one of the two most similar to *S. sempervirens* in their reproductive characteristics, namely, *Sequoiadendron* or *Metasequoia.* Of these two, *Metasequoia* seems to the present writer the most likely. If we imagine a species which, when its characters were combined with those of *Sequoiadendron,* would yield a plant resembling *S. sempervirens,* this imaginary species would possess the following characters: leaves rather large, broad, flat, needle-like, deciduous or with pronounced winter buds; ovulate cones very small, not over 1 cm long, and with 10–12 cone scales, each bearing 3 or 4 ovules; embryos dicotyledonous. This plant would have needles something like those of *Cunninghamia,* although smaller, and cones somewhat similar to those of *Chamaecyparis.* A plant like this seems unlikely to be found either in the living or fossil condition. On the other hand, if we postulate *Metasequoia* as one diploid ancestor of *S. sempervirens,* the other parent would have characteristics about as follows: leaves evergreen, spirally arranged, acicular and pointed, somewhat glaucous; winter buds absent; microsporangiate and ovulate cones terminal on the branchlets; ovulate cone scales with rather long prominent spines; mature cones about 12–15 mm long and half as broad; seeds with narrow, dark wing margins. This combination of characters is by no means an unusual or unexpected one; it is approached from various directions by *Sequoiadendron, Taiwania,* and *Athrotaxis.* There are grounds, therefore, on which to erect the working hypothesis that *S. sempervirens* originated as an allopolyploid from hybrids between an early Tertiary or Mesozoic species of *Metasequoia* and some probably extinct type of taxodiaceous plant not unlike the three modern genera mentioned above.

This hypothesis has value as a speculation, because it can form the basis for certain predictions which will test it. Buchholz (*2*) has pointed out that the embryology of *Sequoiadendron* is somewhat like that of *Athrotaxis* (and perhaps *Taiwania,* which appears not to have been studied), while that of *Sequoia* contains several peculiarities not found in any other genus of the family. On the basis of our present hypothesis, *Metasequoia* should also have these peculiarities (see summary in *3*). Furthermore, if the hypothesis is correct, then among the Mesozoic or early Tertiary fossils of Taxodiaceae which occur associated with *Metasequoia* there should be some which at least approximate the description given above. At any rate, the possibility that the "dawn redwood," *Metasequoia,* may actually be a direct descendant of an ancient ancestor of the present California redwood is a plausible and most intriguing one.

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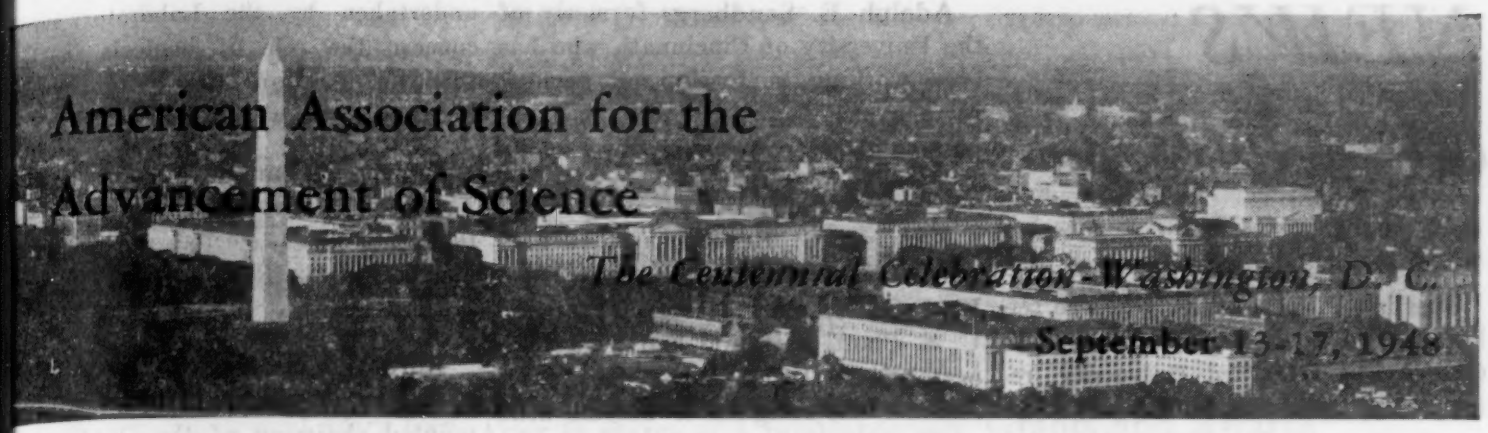

The Carnegie Institution of Washington

The Carnegie Institution of Washington, which will serve as a host institution during the AAAS Centennial Celebration, is an agency engaged in varied programs of scientific research. Founded on January 28, 1902, by Andrew Carnegie, it applies resources of about $44,000,000, in the words of its Articles of Incorporation, "to encourage, in the broadest and most liberal manner, investigation, research, and discovery, and the application of knowledge to the improvement of mankind."

The Institution, which is independent of other Carnegie agencies established at different times for other purposes, is essentially an operating organization. Its objectives and problems are considered and its work is reviewed annually by the Board of Trustees, which meets in December of each year. During the intervals its affairs are conducted by an Executive Committee chosen by and from the Board of Trustees and acting through the president of the Institution, Dr. Vannevar Bush. Standing committees of the Trustees consult with the president concerning research activities in various fields.

The Institution attempts to advance fundamental research in fields not normally covered by the work of other agencies and to concentrate its attention on specific aspects of large problems, with the idea of shifting the approach from time to time to meet the more pressing needs of research as they develop with increase of knowledge.

At present, the principal activities of the Institution are in astronomy, centered at the Mount Wilson Observatory, Pasadena, California; in the biological sciences, comprising the Division of Plant Biology located at Stanford University, Palo Alto, California, the Department of Embryology at Baltimore, Maryland, and the Department of Genetics at Cold Spring Harbor, Long Island, New York; in aboriginal and post-Columbian American history and the history of science, centering in the Division of Historical Research at Cambridge, Massachusetts; and in the terrestrial sciences, centered in the Geophysical Laboratory at 2801 Upton Street and the Department of Terrestrial Magnetism at 5241 Broad Branch Road in Washington, D. C.

Academy Conference

The 1948 Academy Conference, held in connection with the Centennial Celebration of the AAAS at Washington, will include discussions of the following topics: "Federal Aid for Science," by Frank Thone, Washington Academy; "The Academy and the Conservation of Natural Resources," by J. M. Aikman, Iowa Academy; "Cooperation With Industry and Other Scientific Organizations, The Collegiate Academy of Science," by James L. Liverman, Texas Academy. The annual Conference Dinner, sponsored by the AAAS, will be addressed by K. Lark-Horovitz, general secretary of the Association. The meeting will be held on Friday, September 17.

Sigma Delta Epsilon Meeting and Tea

Sigma Delta Epsilon, graduate women's scientific fraternity affiliated with the AAAS, was established May 24, 1921, at Cornell University. Its motto is "United in Friendship Through Science," and its object is to further science, to provide a society for recognition of women in science, and to bring them together in fraternal relationship. In the 27 years since its founding its total membership has increased from a "handful" to 3,500 persons. At present, 14 chapters are spread throughout the United States, and there is one Alumnae Chapter for women in localities with no chapter.

The annual Sigma Delta Epsilon meeting and tea will be held on September 13 between 4:00 and 6:00 P.M. at the Barker Hall, Y. W. C. A., on 17th and K Streets, N. W. Plans have been made to install a new Washington Chapter and to initiate two alumnae members, after which an outstanding speaker will address the group and refreshments will be served. Tickets will be available at the registration desk for 75¢. All graduate women in science are invited.

NEWS and Notes

Sterling P. Fergusson, research fellow at the Blue Hill Meteorological Observatory, Harvard University, retired July 1 in his 80th year, after nearly 61 years of professional meteorological work at Blue Hill (40), the University of Nevada (6), and the U. S. Weather Bureau in Washington (15).

Thorbergur Thorvaldson retired July 1 as head of the Department of Chemistry, University of Saskatchewan, a position he had held since 1919. **John W. T. Spinks,** staff member of the University, has been appointed the new head of the Chemistry Department. Dr. Thorvaldson will continue as dean of the College of Graduate Studies.

Gerhard P. Hochschild, formerly on the staff of Harvard University, has been named assistant professor of mathematics at the University of Illinois.

Alfred H. Meyer, of Valparaiso University, and **Henry M. Leppard,** of the University of Chicago, are currently engaged as visiting professors in geography at the summer session of the University of California at Los Angeles.

Zaboj V. Harvalik, associate professor of physics, School of Mines and Metallurgy, University of Missouri, Rolla, was recently appointed professor of physics, Institute of Science and Technology, as well as research associate at the ORDARK project, University of Arkansas. Dr. Harvalik will assume his new duties in September.

Myron L. Simpson, assistant professor of biology at Gettysburg College, Gettysburg, Pennsylvania, has resigned his position to accept appointment as professor of biology and chairman of the department at Westminster College, New Wilmington, Pennsylvania, effective in September.

Adolph E. Sandberg, formerly of the University of Cincinnati, who has been working in foreign oil geology for the past few years, has been appointed to a professorship in geology at Louisiana State University.

Kenneth C. Beeson, specialist in soils and fertilizers, has succeeded **Karl C. Hamner** as director of the U. S. Plant, Soil, and Nutrition Laboratory at Ithaca, New York. Dr. Hamner has resigned to become head of the Department of Botany at the University of California, Los Angeles.

Chester N. Frazier, Edward Wigglesworth professor of dermatology and syphilology at Harvard University Medical School and director of the Dermatology Service of the Massachusetts General Hospital, has been appointed visiting professor of dermatology and syphilology at the University of Texas Medical Branch, Galveston. He will supervise research work on the etiology of cardiovascular syphilis and residency training in dermatology and syphilology at the John Sealy Hospital.

Emil Witschi, who has been granted a leave of absence from the Department of Zoology, State University of Iowa, for 1948–49, will serve as visiting professor at the University of Tuebingen in the French occupation zone of Germany. The professorship is sponsored jointly by the General Education Board of the Rockefeller Foundation and the University of Iowa.

Conrad B. Link, of the Brooklyn Botanic Garden, has been appointed professor of floriculture in the Department of Horticulture at the University of Maryland and will be in charge of teaching and research in ornamental horticulture. **Pardon W. Cornell,** formerly of the University of Massachusetts, has been made associate professor of ornamental horticulture in the same department.

Stanislas F. Snieszko has been made director of the Microbiological Laboratory, U. S. Fish and Wildlife Service Experiment Station at Leetown, West Virginia. He is now engaged in investigations on methods of treatment and control of fish diseases. One of the next investigations to be undertaken by the Laboratory will concern the role of bacteria in the turnover of organic and inorganic material in fresh waters.

Robert Cushman Murphy, of the American Museum of Natural History, has been elected an honorary member of the Royal Society of New Zealand.

Rufus Oldenburger, of Illinois Institute of Technology, has been appointed chairman of the Department of Mathematics at DePaul University, Chicago.

John P. Hubbard, who has directed a Study of Child Health Services for the American Academy of Pediatrics since 1945, has been named assistant professor of pediatrics at the University of Pennsylvania's School of Medicine. In his new position Dr. Hubbard will be simultaneously engaged in conducting a nation-wide program for the improvement of child health for the Academy and a rheumatic fever teaching program for the Pennsylvania State Health Department.

Austin A. Dodge, who has been with the Philadelphia College of Pharmacy and Science since 1941, has been appointed professor of pharmaceutical chemistry at the University of Mississippi, effective September 1.

Trawick H. Stubbs, associate professor of preventive medicine and community health at Emory University, will become dean of the University of Missouri School of Medicine September 1 upon the retirement of Dudley S. Conley.

George F. Branigan, of Iowa State College, has been named dean of the University of Arkansas College of Engineering, succeeding **George Patrick Stocker,** who has retired with emeritus rank.

Marcus Martin Rhoades, professor of botany at Columbia University, has joined the staff of the University of Illinois. Dr. Rhoades has recently been conducting a seminar in genetics at the Sorbonne.

Carl L. Anderson, formerly associated with the Veterans Administration in Philadelphia, has been ap-

pointed to the Mental Hygiene Division of the U. S. Public Health Service to serve as consultant in clinical psychology to District Office No. 3 in Chicago.

William B. Castle, professor of medicine at the Harvard Medical School and associate director of the Thorndike Memorial Laboratory since 1932, has been named director to succeed **George R. Minot,** who retires. Dr. Castle has for several years been closely associated with the work of his predecessor, who in 1934 received the Nobel Prize in medicine and physiology jointly with William P. Murphy and George H. Whipple for discoveries in the treatment of anemias.

Arno Emerson Town, clinical professor of ophthalmology at New York University's School of Medicine, was appointed professor of ophthalmology at the Jefferson Medical College of Philadelphia on July 1. He succeeds **Charles E. G. Shannon,** who retired on that date.

Maxwell K. Goldstein, electronics engineer who has been on the staff of the Naval Research Laboratory for almost 10 years, has been named to organize and direct the Programs Research Unit of the Research Group, Office of Naval Research. This new office will apply scientific and operational research methods to planning and evaluating ONR's applied research programs. Dr. Goldstein has had wide experience in the development of automatic remote indicating systems for antiaircraft gun control apparatus, radio navigation aids, and radio direction finders.

Bernhard Kummel, geologist with the Bureau of Economic Geology, University of Texas, has been appointed assistant professor of geology at the University of Illinois, where he will teach megascopic invertebrate paleontology and Mesozoic stratigraphy. He will begin his duties in September.

Isadore Rudnick, of Pennsylvania State College, and **Harold K. Ticho,** of the University of Chicago, have been appointed assistant professor and lecturer, respectively, in the Physics Department, University of California, Los Angeles.

Melvin H. Kinsely, of the University of Chicago, has joined the staff of the Medical College of South Carolina, Charleston, as professor and chairman of the Department of Anatomy. Additional departmental appointments include **Elsie Taber,** also of the University of Chicago, who will serve as assistant professor of anatomy and be principally concerned with the fields of embrology and endocrinology, and **Frank Brooks,** formerly of the U. S. Army in charge of a psychiatric unit at the Veterans Administration Hospital at Knoxville, Iowa, as assistant professor of anatomy.

H. Houston Merritt, formerly chief of the Division of Neuropsychiatry at Montefiore Hospital, has become professor of neurology and executive officer of the Department of Neurology of the Faculty of Medicine of Columbia University and director of the neurological service and attending neurologist at the Presbyterian Hospital. Dr. Merritt has done extensive research in the field of epilepsy.

Visitors to U. S.

Alan Fletcher, lecturer in applied mathematics, University of Liverpool, has arrived to visit the special library of Mathematical Tables at Brown University.

Otto Heinrich Warburg, winner of a Nobel Prize in 1931 for his work on cancer and the enzymes of respiration, has recently come from Germany to serve as visiting professor at the University of Illinois. Dr. Warburg is best known in this country for his work on sources of energy for the growth of tumors in rats and for his investigations in the field of photosynthesis.

R. N. Robertson, of the Division of Food Preservation, Australian Council for Scientific and Industrial Research, will be in the United States for the next month. His special interests are plant physiology and fresh fruit and vegetable storage.

Sir Chandrasekhara Venkata Raman, who recently retired from the Bangalore Research Institute to work at the Raman Research Institute, which he has founded in Bangalore, has arrived to attend a meeting of the Advisory Council of the International Bank of Reconstruction in Washington, D. C., and to take part in the International Congress of Crystallographers, which is being held at Harvard this week. His discovery that light, when scattered, changes in wave length and color, known as the Raman effect, won him the Nobel Prize in 1930.

Grants and Awards

The Jane Coffin Childs Memorial Fund for Medical Research recently announced the appropriation of $84,275 for support of various cancer research projects and fellowships. Recipients of the grants include: L. C. Strong, Yale University School of Medicine, supplement of $500 for continued genetical investigations of cancer; Samuel C. Harvey, Yale University School of Medicine, $3,000 for statistical, and $8,500 for clinical and laboratory, studies of cancer; C. C. Little, Roscoe B. Jackson Memorial Laboratory, $4,500 for study of the mammary tumor inciter and related problems; Eugene L. Opie, Rockefeller Institute for Medical Research, $6,000 for cytochemical studies of hepatic changes induced by azo dyes; Harold P. Rusch and James A. Miller, University of Wisconsin, $4,500 for studies on the metabolism of carcinogenic azo dyes and the effect of chemical structure on their activity; Paul R. Burkholder, Yale University, $3,600 for studies of induction mechanisms and propagation of atypical growth in plants; Tracy M. Sonneborn and Willem J. van Wagtendonk, Indiana University, $8,200 for investigations of "paramecin"; William B. Atkinson and Howard C. Taylor, Jr., Columbia University, $4,100 for cytochemical investigations on hyperplastic and malignant uterine tissues in women in relation to their endocrine state; Edward L. Tatum, Stanford University, $15,000 for studies on gene mutation in microorganisms in relation to enzyme production and activity; Edward W. Shrigley, Yale University School of Medicine, $4,000 for biological studies of the Rous sarcoma virus; Frances L. Haven, University of Rochester School of Medicine & Dentistry, $3,000 for studies on storage

and mobilization of fat in tumor-bearing rats; and Gray H. Twombly, Columbia University, $5,000 for study of selective localization of hormones in normal and cancer-bearing tissues by radioactive estrogens. Yale's School of Medicine received $1,000 and $500 for maintenance of the animal house and expenses of seminars, respectively.

Fellowship awards were made to: Joseph E. Sokal, supplement of $1,000 to work with H. S. N. Greene, Yale University School of Medicine; Edward T. Krementz, $3,000 to work with Samuel C. Harvey, Yale University School of Medicine; Ernest Schwartz, $1,500 to work with Samuel Graff, College of Physicians and Surgeons, Columbia University; and Helen P. Thompson, $3,125 to work with K. R. Porter, of the Rockefeller Institute for Medical Research.

The Eötvös Medal, established this year by the Hungarian Academy of Science in celebration of the 100th anniversary of the birth of the Hungarian physicist, Lóránd Eötvös, has been awarded to J. Barnóthy and M. Forró for their contributions to the field of cosmic radiation.

The Charles Goodyear Medal of the American Chemical Society's Division of Rubber Chemistry was awarded on July 23 to David Spence, of Pacific Grove, California, internationally known authority on guayule rubber. Dr. Spence, who received the medal "for his scientific contributions to the growth of the guayule shrub and the production of rubber derived from it," served as a consultant on rubber problems to the Government in both World Wars. Since 1931 he has been acting as consultant and doing experimental work at the Jacques Loeb Laboratory of Stanford University.

Alan W. Bernheimer, assistant professor of microbiology, New York University College of Medicine, received the Eli Lilly Award in Bacteriology at the meeting of the Society of American Bacteriologists in Minneapolis on May 12. Dr. Bernheimer was cited for contributions to the knowledge of bacterial toxins. The award consists of a medal and a $1,000 honorarium.

The 1948 Levy Medal of the Franklin Institute will be presented to Jan A. Rajchman and William H. Cherry, both of the RCA laboratories, at the Institute's Medal Day ceremonies on October 20. The award is given in recognition of their paper, "The Electron Mechanics of Induction Acceleration," which appeared in the April and May 1947 issues of the *Journal of the Franklin Institute.*

Fellowships

The American Physiological Society announces the availability of The Porter Fellowship in Physiology for the academic year 1948–49. The fellowship, carrying a stipend of $2,400 per year, is open to qualified physiologists, preferably at the postdoctoral level. Applications should be made to M. O. Lee, Executive Secretary, American Physiological Society, 2101 Constitution Avenue, Washington 25, D. C.

A fellowship in the Department of Biology, Harvard Graduate School of Arts and Sciences, has been established from a fund provided by the American Creosoting Company. The fund will be used for support of research on the fundamental aspects of the deterioration of wood by fungi and other destructive organisms. The holder will receive an annual stipend of $2,000 and will be expected to pay the usual $525 yearly tuition. The successful candidate will be subject to the general requirements for the Ph.D. in the Department, and, at the end of his first year, the Graduate Committee will determine whether or not the candidate continues for completion of his Ph.D. While the research is to be fundamental in nature with no restrictions by the donors as to scope or content, the holder must give the American Creosoting Company the first opportunity to secure his service if he chooses to go into industrial research.

While a background in mycology, wood structure, physiology, and chemistry is desirable, in selection of the winning candidate more weight will be given his initiative interest, and potentialities for productive research. Candidates should apply on forms obtainable from the Graduate School of Arts and Sciences, Farlow House, Harvard University, before August 15, 1948, so that selections can be made to enable the holder to begin work on September 27, 1948. Candidates will be notified of results not later than September 6.

Colleges and Universities

A cooperative study on poliomyelitis has been undertaken by Columbia University College of Physicians and Surgeons, the Neurological Institute of New York City, and the University of Texas Medical Branch. P. R. Harrington, of the Jefferson Davis Hospital, Houston, will also participate in the study, which has the purpose of devising satisfactory criteria for evaluating the neuromuscular status of poliomyelitis in patients. Investigations will be made on clinical chemotherapy, with special reference to antibiotics and synthetic compounds which have shown promise on experimental trial. The program will be directed by Murray Sanders, of the College of Physicians and Surgeons, C. C. Grulee, Jr., of the University of Texas Medical Branch, and S. Korey, of the Neurological Institute. A conference on the chemotherapy of poliomyelitis, to be held under the auspices of the New York Academy of Sciences, has been arranged for August.

A spectrographic laboratory is being installed at Indiana University by the Division of Geology of the State Department of Conservation. The laboratory will furnish analytical data on limestone, dolomite, sand, gravel, clay, coal ash, and other nonmetallic raw materials. The first program of analysis will include limestones and dolomites of Indiana. Work will be directed by Richard K. Leininger, formerly of the Research Laboratories, Armco Steel Corporation, Middletown, Ohio.

The Graduate School of the University of Tennessee has announced that its Department of Botany will now offer studies leading to the Ph.D. degree. The fields of botanical investigation represented by the training and research interests of the staff are:

bryology, cytology, ecology, genetics, morphology, mycology, pathology, physiology, and taxonomy. Further information may be obtained by addressing the Department of Botany, University of Tennessee, Knoxville 16, Tennessee.

The Hitchcock Museum of Natural History at Amherst College, which will be open to the public this fall, will feature an exhibit which has recently been developed by the Geology Department. The device used for the exhibit, which was designed by George W. Bain, head of the Geology Department, and Theodore Soller, of the Physics Department, will permit a visitor to the Museum to press a button and see and hear a travelogue on some area of the world of current interest. From his extensive collection of color photographs showing landscapes and geological formations and phenomena both here and abroad, Dr. Bain has arranged slides in a series of topics, each concerned with a specific area or region. Through the use of a wire recorder, a running commentary is provided for each series.

The University of Southern California College of Aeronautics at Hancock Field, Santa Maria, California, has announced its new curricula in the field of aviation education. Four-year curricula will be offered in aeronautical engineering (B.E. degree) and air transportation (B.S. degree), and shorter courses will provide training in commercial aviation and airplane and engine mechanics and for pilot certificates and flight instructor and airline transport ratings. The College is under the direction of Allan Hancock.

A Postgraduate Course in Cardiology will be held in Mexico City at the National Institute of Cardiology of Mexico August 2–13, under the sponsorship of the American College of Physicians. The course, unique in that it offers a combined vacation and postgraduate study period, will be a review of the fundamental field of cardiology with especial reference to the work being done in the National Institute of Cardiology. The ACP is also arranging corresponding postgraduate courses to be held in the fall covering internal medicine, endocrinology, cardiovascular disease, cardiology, and gastroenterology.

In addition to its director, Ignacio Chavez, professor of medicine at the University of Mexico and director of the Institute, the faculty will include George R. Herrmann, professor of medicine at the University of Texas, and Edward L. Bortz, associate professor of medicine, University of Pennsylvania, acting as guest instructors, and various members of the staff of both the Institute and the University of Mexico School of Medicine.

Further information about the remaining courses in the program may be obtained by writing to E. R. Loveland, Executive Secretary, The American College of Physicians, 4200 Pine Street, Philadelphia.

The Cornell Aeronautical Laboratory, Inc., until recently operated by the University through the Cornell Research Foundation, Inc., will continue its aviation research as a separate, autonomous unit. The principal reason for reorganization of the Laboratory was to clarify business relations with the University and the Government. Its nonprofit research facilities will continue to be available to the eastern aviation industry.

Meetings and Elections

The Fisheries Institute will convene August 9–14 at the Robert Richter Hotel, Miami Beach, under the sponsorship of the Marine Laboratory of the University of Miami, in cooperation with the U. S. Fish and Wildlife Service, the Florida State Board of Conservation, the Commercial Fisheries Association of Florida, and the Florida Wildlife Federation, together with the fishing industry and other conservation groups in the southeastern states and the Gulf and Caribbean areas. In addition to the presentation of papers, the program will include several round-table discussions: "The Need of a Central Clearinghouse for Fisheries Information in the Caribbean," "The Future of United States Fishery Enterprise in the Caribbean," "Employer-Employee Relations," "Decentralization of Fisheries Research and Federal Support at the State Level," and "How May the Conflicting Interests of Sports and Commercial Fishermen Be Resolved?" Requests for information should be directed to the Fisheries Institute, Marine Laboratory, University of Miami, University Branch, Coral Gables, Florida.

The Field Day of the Connecticut Agricultural Experiment Station will be held this year on August 18 at the Experimental Farm in Mt. Carmel, Connecticut. The theme of the Field Day, "How Science Improves Agricultural Crops," will be developed by the featured addresses by E. C. Stakman, of the University of Minnesota, president-elect of the AAAS, and J. G. Horsfall, director of the Station. The exhibit will be one continuous display of scientific improvement of crops, using the bean plant as an example. Visitors may inspect all experimental plots and fields at the Station and will have an opportunity to learn about current research projects now in progress.

The American Society of Agronomy and the **Soil Science Society of America** are holding their annual meetings at Fort Collins, Colorado, August 24 through 27.

The American Association of Blood Banks will hold its first annual meeting at the Hotel Statler, Buffalo, New York, August 26–28. Information about the Association and the meeting may be obtained from the Office of the Secretary, 3301 Junius Street, Dallas 1, Texas.

A regional conference on applied statistics has been scheduled for September 7–9 at the Statistical Laboratory, Alabama Polytechnic Institute, Auburn. This conference, which is being held under a grant to the Statistical Laboratory from the General Education Board, will include programs allocated to papers on the application of statistical methodology to research in the social, plant, and animal sciences. Statisticians who will appear on the program include: George W. Snedecor, Gertrude M. Cox, Earl Houseman, Boyd Harshbarger, R. L. Anderson, H. L. Lucas, J. A. Rigney, and T. A. Bancroft. Brief research reports involving statistical methodology will also be presented by

research workers drawn mainly from the southeastern states.

The 30th summer meeting of the Mathematical Association of America, to be held at the University of Wisconsin, September 6–7, will include the following speakers: first session—W. H. March, University of Wisconsin and U. S. Forest Products Laboratory, "Mathematical Problems Associated With the Use of Laminates in Aircraft"; S. S. Cairns, University of Illinois, "Definition of Angles in Higher Dimension Spaces"; H. F. S. Jonah, Purdue University, "An Experiment in Teaching Large Classes in Mathematics"; second session—J. B. Rosser, Cornell University, "Exterior Ballistics of Artillery Rockets"; Samuel Eilenberg, Columbia University, "What Is Homotopy?"; and M. H. Stone, University of Chicago, "Enrichment of the Mathematical Curriculum for Juniors and Seniors."

A Gas Discharge Conference, to be held at the Brookhaven National Laboratory on October 27–29, is being arranged by a committee consisting of L. H. Fisher, New York University (chairman), W. P. Allis, Massachusetts Institute of Technology, J. B. H. Kuper, Brookhaven National Laboratory, and J. P. Molnar, Bell Telephone Laboratories, acting in cooperation with the Laboratory. Discussion is expected to be organized around four main topics: Fundamental processes in the gas and at the electrodes; Breakdown phenomena—DC and microwave; Counter tubes—physical processes (not circuitry); and Operating discharges—glows, arcs. Papers will be welcomed. Further information may be obtained from Mrs. M. K. Kuper, Executive Aide, Director's Office, Brookhaven National Laboratory, Upton, New York.

The Virginia Academy of Science held its 26th annual meeting in Roanoke on May 6–8 with Virginia Polytechnic Institute as host and Jesse W. Beams, professor of physics at the University of Virginia, presiding. Approximately 660 Seniors and 150 Juniors were in attendance. There were 170 papers presented in the 10 Sections of the Senior Academy and annual reports were submitted by chairmen of 15 permanent committees. The Junior Academy was addressed by A. N. Vyssotsky, of the University of Virginia, and George W. Jeffers, of Farmville State Teachers College. Officers elected by this group were Paul Thompson, Grundy, president-elect, and W. Herman Bell, Jr., Norfolk, secretary. Beverly S. Parrish, Jr., Martinsville, was installed as president for 1948–49.

Henry Leidheiser, Jr., associate in chemistry at the University of Virginia, was awarded the J. Shelton Horsley Research Prize for his paper, "Generalizations Concerning the Surface Behavior of Single Crystals of the Face-centered Cubic Metals." Research grants totaling $1,070 were made to Trent Laviana, Richard L. Hoffman, Percy C. Holt, and Marvin L. Bobb, of the University of Virginia; Edwin W. King and Wilson B. Bell, of Virginia Polytechnic Institute; Robert B. Platt, of the University of Pennsylvania; Zoe W. C. Black, of Mary Washington College; and R. F. Sessions, of the University of Richmond.

E. C. L. Miller, secretary-treasurer for 25 years, was presented by his friends a portrait of himself painted by Herbert E. Ives, physicist. The 15 winners of the 3rd Virginia Science Talent Search were announced. All have received college scholarships for the coming year. They are: William E. Atkinson, Richard E. Lyle, and Mary Ann DuVal, of Thomas Jefferson High School, Richmond; Bernard M. Brown and Paul L. Goodfriend, of Granby High School, Norfolk; Algar H. Cosby and Bertram C. Taurman, of John Marshall High School, Richmond; George P. McCasland, James B. Sutphin, and Anne Kerfoot Brown, of Lane High School, Charlottesville; Julien A. Scott, Jr., of Northampton High School, Eastville; Ashby W. Spratley, of Maury High School, Norfolk; Millard C. Townsend, of William Fleming High School, Roanoke; Paul G. Von Beck, of Warren County High School, Front Royal; and Elizabeth B. Bush, of William Byrd High School, Vinton.

Awards were also made to winners of Junior Academy exhibits, and two science club sponsors, Miss Vera Baron, of Martinsville, and Mrs. B. G. Heatwole, of Fisherville, were presented summer school scholarships a awards for their inspirational teaching of high school science. The Wilso Memorial High School Science Club of Fisherville, won the E. C. L. Mille Award of $50 for having carried ou during the year the best club progra of all 150 clubs in the State.

It was announced that during th year $28,000 had been appropriate by the State Legislature for Academ projects—$20,000 for helping to ge under way the Virginia Institute fo Scientific Research and $8,000 fo publishing a monograph on the Jame River basin. Gifts of $3,000 for cu rent expenses of the Academy an $1,160 for work among "junior scientists" were announced. It was reported that the membership of th Academy now approximates 1,100.

For the first time the AAAS dis played an exhibit at a state academ meeting. About half of this exhibi was given over to Virginia Academ of Science material. The researc committee of the Academy sponsored demonstration of phase contrast mi croscopy by Oscar W. Richards, chie biologist of the American Optica Company. Some 35 exhibits were dis played by members of the Junio Academy.

Following the annual banquet, panel discussion arranged by E. S C. Handy was held on "The Socia Responsibilities of Scientists in Unique World." John Collier, presi dent of the Institute of Ethnic Affairs was discussion leader. Geological biological, and industrial field trip were participated in by over 150 per sons.

Officers elected for 1948–49 were Boyd Harshbarger, president-elect; E C. L. Miller, secretary-treasurer; Foley F. Smith, assistant secretary-treas urer; and Guy W. Horsley, new mem ber of the Council. Sidney S. Negus chairman of the Chemistry Depart ment at the Medical College of Vir ginia, was installed as president fo 1948–49.

Richmond was selected as the meet ing place for next year, the meeting being scheduled for May 5–7.

The Colloquium of College Physi cists held its 9th annual meeting a

the State University of Iowa on June 10, 11, and 12. G. W. Stewart, professor of physics at Iowa, reports that 125 college physicists attended from 18 states and 70 colleges. Two dozen of them contributed to the exhibit new devices for lecture room and laboratory instruction, and much enthusiasm was shown over this creative work of the members. First, second, and third prizes were awarded, respectively, to Jerome Brewer, Midwest Research Institute, Kansas City, Missouri; Z. V. Harvalik, University of Missouri, Rolla, Missouri; and W. D. Bemmels, Ottawa University, Ottawa, Kansas.

The program consisted of reports on current researches in nuclear physics, origin of cosmic rays, and upper atmospheric research, presented, respectively, by Joseph M. Keller, of Iowa State College; W. W. Salisbury, director of research, Collins Radio Company; and James A. Van Allen, of the Applied Physics Laboratory, Johns Hopkins University. There were round-table discussions on scientific method, Princeton Conference on Science in General Education, and methods employed by 20 colleges in physics courses in general education. Dinner addresses were given by Dean Earl J. McGrath, on the function of science in general education; W. W. Waymack, of the Atomic Energy Commission; C. N. Wall, on comparisons of teaching opportunities in colleges and large universities; and Charles H. Schauer, of the Research Corporation.

The closing session on atomic energy control was participated in by Dean Louis N. Ridenour, C. Rogers McCullough, and W. W. Waymack.

The next meeting will be in June 1949.

The International Association for Hydraulic Structures Research, at its meeting in Stockholm, Sweden, June 7–9, selected Grenoble, France, as the place for its meeting in August 1949, preceding the conference of the International Congress of Navigation, which will be held in Lisbon. Delhi, India, was selected as the meeting place of its next triennium conference in February 1951.

The newly elected officers, whose terms are effective on January 1, 1949, are as follows: president, Lorenz G. Straub, U. S. A. (Minneapolis); 1st vice-president, E. Meyer-Peter, Switzerland; 2nd vice-president, A. N. Khosla, India; secretary, J. Th. Thijsse, The Netherlands. Permanent Committee members include, in addition to officers, G. De Marchi, Italy; P. Danel, France; and Sir Claude Inglis, England. Corresponding Members are B. Hellstrom, Sweden, and M. Bayer, Czechoslovakia. Wolmar Fellenius, the retiring president, was elected Honorary Member and ex-officio member of the Permanent Committee.

Local Sections of the Optical Society of America have recently held their annual elections. Some of these have been reported by Stanley S. Ballard, secretary of Local Sections of the Society, as follows: Rochester, New York—John H. McLeod, Hawk-Eye Works, Eastman Kodak Company, president; H. D. Polster, University of Rochester, vice-president; J. R. Benford, Bausch & Lomb Optical Company, secretary; and L. T. Steadman, University of Rochester, treasurer; Detroit, Michigan—David L. Fry, General Motors Research Laboratories, chairman; Karl W. Beyer, Pennsylvania Salt Manufacturing Company, vice-chairman; Daniel L. Harmon, University of Detroit, secretary; and W. R. O'Neill, Ethyl Corporation, treasurer; Niagara Frontier (Buffalo, Niagara Falls, and vicinity)—Galen Porter, Union Carbide and Carbon Company, president; Leland E. Havens, Durez Plastics & Chemicals Company, secretary; and Clair M. Birdsall, Linde Air Products Company, treasurer.

The new Ohio Valley Section of the Society was recently installed at the Charles F. Kettering Foundation, Antioch College, Yellow Springs, Ohio. Dr. Ballard, installation officer, spoke on this occasion on "Spectroscopy and Radiometry at Bikini." This section, which has a membership of about 50, was organized as an outgrowth of the Ohio Valley Spectrographic Society, founded in 1944 to serve interested groups in the area. The meetings will be distributed about equally among Cincinnati, Middletown, Dayton, and Yellow Springs. Officers of the new Section are: M. Eugene Merchant, Cincinnati Milling Machine Company, chairman; J. F. Woodruff, American Rolling Mill Company, chairman-elect; C. S. Mills, also of Armco, secretary-treasurer; and C. H. Aneshansley, National Cash Register Company, Ivan T. Collier, Moraine Products Division, General Motors Corporation, Wendell R. Koch, Materials Laboratory, Wright Field, and Richard Olt, Monsanto Chemical Company, councilors.

NRC News

The Committee on Growth, acting for the American Cancer Society, is entertaining applications for grants and fellowships. Applications for extension of existing Grants in Cancer Research will be received until October 1; applications for new grants, until November 1. Final decision on applications submitted during this period will be made in most cases soon after February 1. Grants approved at this time ordinarily will become effective on July 1, 1949.

Fellowship applications may be submitted at any time. Those received prior to November 1 will be acted upon by the Committee in December. Those received between November 1 and March 1 will be acted upon in April. Fellowships ordinarily will begin on July 1, though this date may be varied at the request of the applicant.

During the past year the American Cancer Society, Inc., on recommendation of the Committee on Growth, has approved research grants and fellowships totaling over $2,000,000.

Communications regarding grants and fellowships should be addressed to Executive Secretary, Committee on Growth, National Research Council, 2101 Constitution Avenue, N.W., Washington 25, D. C.

Deaths

Austin Roberts, of the Transvaal Museum, Pretoria, South Africa, died on May 5. According to word recently received, Dr. Roberts collapsed from heart failure at the wheel of his automobile while on a vacation trip. Dr. Roberts' American friends will be interested to know that his *Mammals of South Africa,* companion volume to the well-known *Birds of South Africa,* is almost ready for publication.

P. L. MacLachlan, 40, professor of biochemistry and head of the department, School of Medicine, West Virginia University, died July 17 of a cerebral hemorrhage.

The Library of Congress is now recruiting a number of qualified people to be engaged in abstracting government scientific and technical reports. The positions carry a salary of $4,479 per annum. Applicants should have a Master of Science degree, but civil service eligibility is not required. Further information may be obtained from the Director of Personnel, Library of Congress, Washington, D. C.

Annual Reviews, Inc., has just announced that an *Annual Review of Psychology* is to be published under its auspices, Volume I to appear early in 1950. The preliminary organization is now complete. Calvin P. Stone and Donald W. Taylor, of Stanford University, will serve as editor and associate editor, respectively, and the Editorial Committee will consist of John E. Anderson, Institute of Child Welfare, University of Minnesota; John G. Darley, University of Minnesota; Clarence H. Graham, Columbia University; Carl I. Hovland, Yale University; and James G. Miller, University of Chicago.

It is intended that in the new *Review* the editorial policies which govern the *Annual Review of Biochemistry,* the *Annual Review of Physiology,* and the *Annual Review of Microbiology* will maintain. The subject matter of each annual volume will consist of critical appraisals of the research proceeding in the major divisions of the field. Subjects of greatest activity will be reviewed annually, while those of lesser activity, together with any topics which encompass small divisions of the field, will be reviewed biennially.

Organization of an *Annual Review of Physical Chemistry* of a similar type, operating under the same editorial policies and characterized by a corresponding recurring list of topics, has also been announced. This *Review,* to be edited by G. K. Rollefson, of the University of California, Berkeley, will be directed in the selection of topics and authors by an Editorial Committee consisting of Henry Eyring, University of Utah; George Glockler, State University of Iowa; W. F. Libby, University of Chicago; J. W. Williams, University of Wisconsin; and E. Bright Wilson, Jr., Harvard University. The first volume of this *Review* will also appear in 1950.

Annual Reviews, Inc., is a nonprofit corporation which was first constituted in 1932 to publish the *Annual Review of Biochemistry.* Since then it has initiated the *Annual Review of Physiology* (jointly with the American Physiological Society) and the *Annual Review of Microbiology.* The members and directors of Annual Reviews, Inc., consist of H. J. Almquist, F. E. Booth~ Company, Inc., Emeryville, California; H. A. Barker, University of California, Berkeley; H. J. Deuel, Jr., University of Southern California; J. F. Fulton, Yale University; D. R. Hoagland, University of California, Berkeley; J. Murray Luck, Stanford University; and H. A. Spoehr, Carnegie Institution of Washington, Stanford, California.

A female specimen of the frilled shark, *Chlamydoselachus anguineus* garman 1884, was caught by Pete Metson, a commercial fisherman of Santa Barbara, on June 30, according to a note received from Elmer R. Noble, chairman of the Department of Biological Science, Santa Barbara College, University of California. The shark was caught in a surface net about 60 miles off Point Arguello, California, where the water is approximately 700 fathoms deep. This appears to be the first record of this species from the coast of North America. The shark has been on display at the Santa Barbara Museum of Natural History and is now at the California Academy of Sciences in San Francisco. Although the shark was eviscerated by the fishermen as soon as it was caught, the liver was saved. Some dimensions are as follows: total length, 1,718 mm; tip of liver to the last gill septem, 260 mm; anus to tip of tail, 655 mm.

The Mexican Government has pledged 20,000 Mexican pesos (slightly more than $4,000) to the International Cancer Research Commission, headquarters for which will be Mexico City during the next three years (see *Science,* April 30, p. 442). According to Rafael P. Gamboa, Minister of Public Health, who made the announcement, the Commission has been enthusiastically approved by both his Department and President Aleman. Invitations have been issued by the Mexican Department of Foreign Affairs to all countries to send delegates to the meeting of the Commission, to be held in Paris October 17–21.

The Atomic Energy Commission has announced the financing of 38 biological and medical research projects in non-governmental institutions, under a joint program with the Office of Naval Research. The $1,300,000 project, to be administered by ONR, will include research programs at the following institutions: Barnard College, Boston University, California Institute of Technology, University of California (Berkeley), University of Chicago, Harvard University and Harvard Medical School, Massachusetts Institute of Technology, Peter Bent Brigham Hospital, Howard University, University of Illinois, University of Kansas, Massachusetts General Hospital, Meharry Medical College, Memorial Hospital for Treatment of Cancer, University of Misssouri, University of North Carolina, Ohio State University, Rice Institute, University of Rochester, Saranac Laboratory, Sloan-Kettering Institute, Southern Research Institute, Tufts College Medical School, Union College, University of Virginia, Wake Forest College, Washington University, Western Reserve University, and Yale University.

Making Plans for—

International Society of Hematology, biannual meeting, August 23–26, Hotel Statler, Buffalo, New York.

Third Symposium on Combustion and Flame and Explosion Phenomena, September 7–11, University of Wisconsin, Madison.

Symposium on Cerebral Mechanisms and Behavior, September 20–25, California Institute of Technology, Pasadena.

Biological Photographic Association, annual convention, September 8–10, Houston Hall, University of Pennsylvania, Philadelphia.

Comments and Communications

The Structure of Antigen Films and Long-Range Forces

Recently, A. Rothen (*Science*, November 2, 1945, p. 446; *J. biol. Chem.*, 1947, 168, 75) has reported experiments involving the interaction between films of spread antigens and homologous antisera. The effects of the deposition of screens of barium stearate, octadecylamine, and Formvar on the antigenic film prior to treatment with antibody were studied. He found that these inert films did not completely prevent the specific immobilization of antibody. After considering and rejecting interpretations of these results in terms of holes in the screens and diffusion of the protein molecules, Rothen has proposed the conception of specific long-range forces, extending over 200 A, to account for his observations.

A basic assumption in Rothen's argument is the claim "that the antigenic films consisted of completely unfolded molecules, since the thickness per monolayer was found consistently between 8 and 9 A after transfer under 8 dynes of pressure" (*J. biol. Chem.*, 1947, 168, 79). It has appeared to us that this claim is unjustified and that, in fact, the antigenic film is not of constant thickness but may contain projections as high as 200 A. On this basis it is possible to account for Rothen's results without the need to assume specific long-range forces. We have carried out electron microscope examination of antigenic films, and the evidence obtained does indeed indicate an irregular structure.

The fallacy in the above quotation, as to the smoothness of the antigenic film, arises from the fact that the method employed by Rothen (*Rev. sci. Instr.*, 1945, 16, 26) determines only an average optical thickness and will not detect irregularities whose dimensions are small compared to the wave length of light. This is clear from the derivation of Drude's equations upon which this method is based (*Theory of Optics*. New York: Longmans' Green, 1913. P. 287).[1] Thus, since visible light was used, it is not excluded that there may have been peaks as high as 200 A in the antigen films.

In attempting to understand how an irregular film can arise from the original compressed monolayer of protein on water, it must be remembered that the protein layers are dried after transfer to the slide and before the adsorption experiment is conducted. The stability of a protein monolayer on water depends upon the interaction between water molecules and the polar side chains of the protein (H. B. Bull. *Adv. prot. Chem.*, 1947, III, 95). On drying, this stabilization effect is lost or greatly reduced. It is therefore necessary to consider the possibility that a refolding of the extended molecules occurs, followed by molecular aggregation or, perhaps, even the formation of microcrystals. The fact that reversible extension of protein molecules in solution occurs is well known, as, for example, in the case of urea-denatured serum albumin (H. Neurath, G. R. Cooper, and J. O. Erickson. *J. phys. Chem.*, 1942, 46, 203).

On the basis of such a picture, it is only necessary to assume that when the barrier films are laid down over the antigen film, the protein peaks project through and can thus interact directly with the antibody. It is to be expected that there will be a distribution in height of these peaks. As a result, the number of peaks exposed per unit area (or, preferably, the area of protein exposed per unit area of slide) would decrease with increasing thickness of blanket. This result is consistent with the experimental relation Rothen found between the amount of adsorbed antibody and the thickness of screen.

Other observations reported by Rothen can readily be accounted for in terms of the interpretation suggested above. For example, conditioning of a slide covered by an optical gage of barium stearate with uranyl acetate increases the number of layers of bovine albumin which can subsequently be deposited. Since such conditioning results in an increase of optical thickness of about 8 A, it would appear likely that there has been a deposition of uranyl salts, probably in the form of small crystals. It might be expected, then, that these crystals would act as polar sites on which the protein would be held and on which folding and aggregation might occur. Further, Rothen found that bovine serum albumin showed much more striking effects than did egg albumin. It appears to us as significant in this connection that bovine albumin can, after denaturation, much more readily revert to a soluble form resembling the native protein than can egg albumin (H. Neurath, J. P. Greenstein, F. W. Putnam, and J. O. Erickson. *Chem. Rev.*, 1944, 34, 243).

As mentioned above, we have sought confirmation of our views of the structure of the dried antigenic film by electron microscope examination. Monolayers of bovine serum albumin were prepared on twice-distilled water in accordance with Rothen's procedure.[2] The protein was then transferred to carefully cleaned glass slides by repeated immersion. The slides were rigidly held in a vertical position and were moved by rack and pinion. The transfer could be followed by the reduction in surface pressure resulting from the removal of protein from the water surface onto the slide. It was found that the transfer occurred only during the withdrawal of the slide from the water, since no change was observed during immersion. It was possible to transfer as many as 6 mono-

[1] Drude's method for measuring film thickness has been subject to question and, recently, refinements in the method have been reported (Lucy. *J. Chem. Phys.*, 1948, **16**, 167). It is reasonable to expect that a film with irregularities which are small compared to the wave length of the light used will cause plane polarized light to become elliptically polarized upon reflection through it, but the effective index of refraction and the relation between the actual mean thickness and the optical thickness given by the method requires further investigation.

[2] We are indebted to Dr. Hayashi, of Columbia University, for assistance in the preparation of the protein monolayers.

layers, a fact which in itself suggests the importance of the polarity of the surface in the retention of the protein. It should be pointed out that our antigen films differ from Rothen's in that his were deposited on an optical gage of barium stearate or octadecylamine.

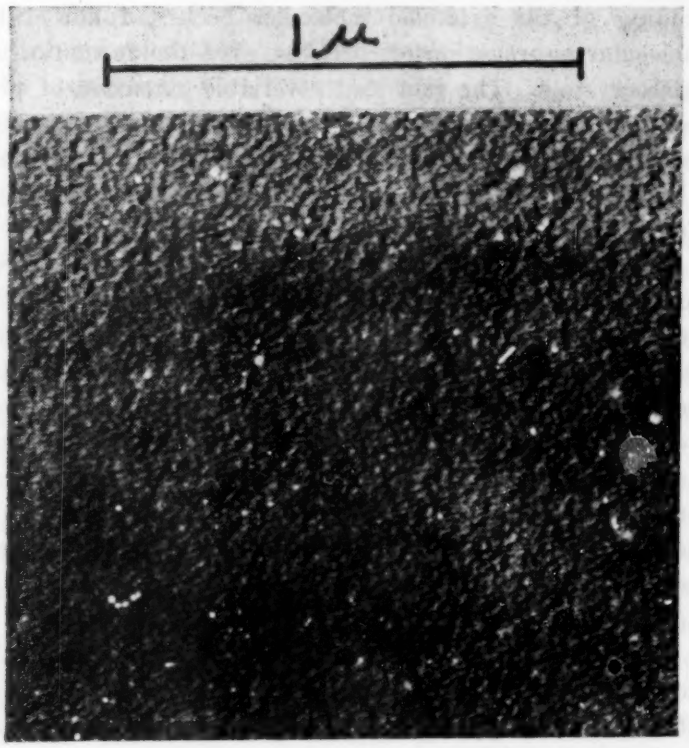

FIG. 1

A direct collodion replica of the protein film was prepared by casting in the usual manner from an amyl acetate solution. The collodion film was stripped and the negative replica obtained was shadowed with chromium at an angle of 1: 5. Fig. 1, representing a magnification of 50,000 ×, shows the appearance of the protein film resulting from the transfer of 6 monolayers of bovine serum albumin. What appear as depressions or pits correspond to ridges or peaks in the original protein film. The heights of these peaks can be calculated from the electron micrograph of the replica by assuming that their slopes are the same on both sides. Such measurements reveal that the projections in this case range generally between 50 and 85 A, with a few greater than 100 A.

In addition, the preshadow replica technique (R. C. Williams and R. W. G. Wyckoff. *J. appl. Phys.*, 1946, 17, 23) was used to prepare a direct positive representation of a slide with one monolayer of protein. Here, too, projections of significant proportions were evident. Control replicas of clean glass slides produced practically structureless micrographs, as expected from collodion cast on glass.

In conclusion, it appears to us that, in view of the considerations and evidence presented above, the claim that the experiments of Rothen establish the existence of *specific* long-range forces is unjustified at this time. It is perhaps unnecessary to remark that because of the fundamental significance of such a conception the most critical appraisal, both theoretical and experimental, of the hypothesis of specific long-range forces should be made before it is regarded as validated.

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Research and the Geographic Factor

In the February 6 issue of *Science* (pp. 127–130) Clarence Mills brought forward a strong indictment of existing methods and institutions concerned with the distribution of funds for research on the basis of discrimination against certain geographic areas by favoritism to others. In the April 16 issue of *Science* (p. 391), Thomas Turner has argued contrariwise to the effect that disbursing bodies are under obligation to place research funds where they believe the most productive immediate results will be achieved and that the results of research benefit not merely a community but the country as a whole. The points which Mills and Turner make are interesting, but both writers imply a certain deliberation in the distribution of funds according to an artificially simplified plan which may be questioned. In the past two years the American Neurological Association has been concerned with the collection of certain information which may be considered to cast some light upon the question of the geographic distribution of research funds. It was the specific intention of the American Neurological Association to determine what agencies had in the past allotted funds for research dealing with the nervous system, or for training in one of the disciplines concerned with that system. The Association was further interested in determining what proportion such aid bore to other aid made by the same agencies, whether financial assistance of this type had proportionally increased or declined, and what institutions and geographic areas had been so aided.

The over-all implications of the survey were clear and in conformity with expectation. The principal contributors to such research and training were governmental agencies, over-all aid had increased absolutely and had remained relatively unchanged (though certain basic fields dealing with the neural system were all but ignored), and most of the aid had gone to the larger and better-known institutions north of the Mason-Dixon line and east of the Mississippi.

The survey disclosed certain factors which have a direct bearing upon the last consideration. The agencies, foundations, and funds able and willing to give an account of their present expenditures were few. Still fewer were able and willing to compare their present with past disbursements. Very few indeed were able and willing to make any kind of a breakdown in terms of the exact disciplines aided. There are several obvious reasons why a comprehensive picture could not be obtained from all the agencies, funds, and foundations contacted. In the first place, the mortality among such organizations is high, and only a few of the better known have been in existence or have held to a consistent policy long enough

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to make comparisons extending over a full decade. In the second place, it is a regrettable fact that some of these bodies are more impressive on paper and in theory than in fact. Finally, there are very few organizations which have really comprehensive records organized in such a way as to yield information other than that which appears in a set form in their annual report. It might be supposed, by a disaffected individual, that a considerable proportion of the agencies, funds, and foundations were deliberately concealing a dubious administrative policy and simply would not yield the information requested. Granting that certain so-called philanthropic foundations are dubious in inception and devious in administration and granting that all offices receive more questionnaires than they have any inclination to answer, the fact remains that all the better-known agencies, funds, and foundations did answer the Association questionnaires to what appeared to be the best of their ability. Since it is obvious that the conclusion that most of the aid was distributed to the northeast came from the answered questionnaires, it is apparent that this aid came from the said larger and better-known organizations.

When this material was examined, certain very interesting observations emerged. In spite of Mills' figures, the older, private philanthropic organizations very evidently made a definite and deliberate attempt to aid research and foster training in areas other than the northeast. It is probable, had the figures been corrected (the records, having been kept confidential, have now been destroyed) for population density and number of educational institutions in given areas, that it would have been found that areas other than the northeast had actually been somewhat favored by such older, private foundations.

In the case of the younger, private foundations and government agencies (most of which date, of course, from the period of the last war) the situation was otherwise. There are several obvious reasons for this. Smaller institutions have a very small factor of safety in personnel. With an accelerated teaching schedule, opportunity for research was wiped out in small faculties. The situation was, of course, worse than that, since the smaller schools actually lost personnel to the armed forces. It can only be hoped that many of these men found their way into government-maintained laboratories. Some certainly did.

Under the circumstances, applications for research and training from many geographic areas failed to appear, and even deliberate efforts by some of the government agencies to encourage research in those areas failed.

Although the war is over, something of the same situation still obtains. The number of bona fide requests for aid for appropriate grants still remains small from certain geographic areas. No one could seriously expect philanthropic organizations, committed to the proposition of developmental research, to make a grant to an institution in a geographic area simply because other geographic areas had received such grants and that area had not. Those individuals who seek to justify the differential in geographic distribution by demolishing such a supposed argument are merely destroying a straw-horse of their own confused construction. Again, one often finds the geographic differential justified on the basis that a grant cannot be made if an application for a grant is not received. In this connection the faculty of a small, outlying institution is in a peculiar position. Its members might well ask: "How can an application be filed if one does not know what is available and where?"

It is upon this question that the American Neurological Association questionnaire throws some light. In the early organizational stages the policies of an agency, foundation, and fund may be extremely nebulous. The organization does not clearly state its policy because it does not clearly comprehend it itself. This is not the fault of the organization. It takes time and experience to develop policies, and it is best not to be too specific until one knows that the way ahead is clear. The requests such a developing organization receives are likely to come from the individuals (or persons associated with those individuals) who are attempting to formulate that policy. This may look like nepotism to the casual observer, but in point of fact it is the inevitable result of a lack of a well-developed and long-range point of view. After all, who knows the future? Before the faculty of an isolated institution becomes aware of the function of a particular governmental agency, that agency's policies may have altered or it may have actually ceased to exist.

How can a given individual in search of information about available fellowships or research grants in a particular field obtain information about these things? The question is not an easy one to answer. Fellowships spring up suddenly, support for particular fields of endeavor ceases. If a directory were prepared, it would be out of date by the time it was printed. If a National Science Foundation is at some time established, a certain more or less stable framework should be provided for at least a basic level of activity in research and training for research. It is the consensus of the Committee on Medical Legislation of the ANA that some of the funds of such a foundation should be allotted on a geographic basis. It should not be necessary to justify allotment of these funds on the basis that the research done with them could not be done better elsewhere. The best-equipped laboratories should not be allowed to compete for such funds. Such funds should be regarded as a developmental investment. If they yield outstanding research, well and good; if not, they will still have served the country as insurance against the possible neglect of a fruitful idea which may have been otherwise overlooked in a certain geographic area.

Although a bill has passed the Senate, no one knows what form a National Science Foundation will take if it is finally established. In the meantime, the best that can be suggested to the individual in search of information as to what fellowships or research funds may be available in a given field is to direct an inquiry phrased in as specific language as possible to the representative of his professional academy, association, or society to the National Research Council, Washington, D. C.

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TECHNICAL PAPERS

Photosynthetic Studies With Mutant Strains of *Chlorella*[1]

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In 1946 experiments were started in this laboratory with the aim of developing a new method for the study of photosynthesis. It was hoped that the method developed by Beadle and Tatum (*1*) with *Neurospora* for the study of physiological genetics could be adapted to green organisms for the study of photosynthesis. If individual reactions in the photosynthetic mechanism of an organism could be blocked through gene mutations, it is possible that intermediate stages in the process could be traced and a series of reactions built up for the process such as Tatum, Bonner, and Beadle (*5*), Bonner (*2*), and Srb and Horowitz (*4*) have done in the study of other biochemical syntheses.

A green alga, *Chlorella* sp., originally isolated and obtained in pure culture from a single cell by Dr. Hempstead Castle, was used in this study. The cells were grown in Moore's solution (*3*) at one-fifth dilution with the addition of trace elements, glucose, and tryptone. Mutant strains were obtained in the following manner: Cells were suspended in quartz tubes in a complete medium containing one-fifth Moore's solution, trace elements, glucose, tryptone, yeast extract, and a vitamin mixture. The suspensions were irradiated with ultraviolet light at a wave length of 2,537 A. The irradiation time was varied, and dilution plates of the irradiated suspensions were made on a solid complete medium containing the same components as the medium in which the cells were irradiated. The plates were placed under continuous light. Only colonies obtained on plates which had 0.1% the number of colonies contained in nonirradiated control plates were used for subsequent studies. Transfers were made from widely spaced colonies to agar slants of the complete medium. Inoculations from the complete slants were then made to minimal liquid medium tubes containing one-fifth Moore's solution and trace elements, in which wild type *Chlorella* grows normally. The presence or absence of growth in the minimal medium was used as the basis for the detection of biochemical mutants. The absence of growth on the minimal medium indicates that the cells are unable to synthesize some component contained in the complete medium. Growth on both the complete and minimal media was used to indicate that a deficiency had not been induced.

Three strains were isolated which have absolute deficiencies, *i.e.* are completely unable to grow on the minimal medium. (These strains shall hereafter be referred to as nongrowers.) Seven others were obtained which exhibit a negligible amount of growth compared with the wild type on the minimal medium. Two of these are also morphological variants. All of these strains are green when grown on the complete medium. The following will deal only with the nongrowers.

An analysis was made of each mutant's ability to grow on media containing the minimal medium plus individual components of the complete medium, *i.e.* glucose, tryptone, yeast extract, and a vitamin mixture. In addition to these, casein hydrolysate and a combination of casein hydrolysate and vitamins were tested. None of these media except that containing glucose supported growth of the three nongrower strains. During the past two years the mutant strains have not regained the ability to exist autotrophically and have continued to remain completely heterotrophic.

Comparative growth studies were made with the wild type and two of the nongrowers. These were grown both in the light and in the dark in minimal solutions containing glucose which was varied from 0 to 2%. The growth of the mutants in the light and dark and of the wild type in the dark was about the same at the lower glucose concentrations. However, the growth of the mutants in the light was superior to that of the mutants and of the wild type in the dark at the higher glucose concentrations. As the glucose supply is depleted, the mutant cultures gradually lose their green color until they become colorless. The green color of such cultures can be restored by the addition of glucose to the medium.

There is evidence to indicate that failure to grow in the minimal medium is not caused by blocks in the production of chlorophyll. The total pigment absorption spectra of the mutants and wild type reveal peaks at identical wave lengths. Further evidence comes from the fact that the cells are green when grown in the presence of glucose. It thus appears that all of the reactions following the synthesis of glucose to the formation of chlorophyll are present and operative.

The mutants were tested for their abilities to liberate oxygen when suspended in a 0.1 molar carbonate-bicarbonate mixture and exposed to light. Oxygen uptake was noted rather than oxygen liberation, which occurs with wild type cells under similar circumstances. The fact that oxygen liberation due to photosynthesis has not been demonstrated for two of the mutant strains suggests that there are blocked reactions in the photosynthetic mechanisms of these mutants.

[1] This work was supported by a fellowship from the F. A. Bartlett Tree Expert Company, Stamford, Connecticut.

[2] The author wishes to express his gratitude to Drs. A. W. Naylor, E. L. Tatum, David Bonner, and P. R. Burkholder for their helpful suggestions and discussions.

Growth studies and gas exchange measurements have revealed differences in the capabilities of the mutant strains. It is probable that different reactions are blocked in each. By determining which reactions are blocked in these mutants, together with similar studies on others, it is possible that a number of intermediate steps in the photosynthetic mechanism can be positively determined.

A more complete report including supporting data will be published at a later date.

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Fractionation of Amino Acids From Hydrolysates in Nonaqueous Systems

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All of the amino acids which are present in an acid hydrolysate of a protein can be brought into solution in acetone by the action of certain organic acids which form with them salts or complexes which are soluble in this solvent. Thus far we have found no other solvent as good as acetone for this purpose. Alcohols react with the reagents, and dioxane, although of possible usefulness in special cases, is less satisfactory than acetone. Methylethyl ketone is greatly inferior to acetone as a solvent for the amino acids with the organic acids which we have tested as reagents. We have explored the possibilities of fractionation of amino acid mixtures in several nonaqueous systems, making use of differences in solubility of individual amino acids in acetone solutions of certain organic acids.

A large number of acids have been examined for their power to cause solution of amino acids in acetone. The following possess this property to a useful degree: trichloroacetic acid, dichloroacetic acid, *p*-toluenesulfonic acid, 4-nitrochlorbenzenesulfonic acid, *d*-camphorsulfonic acid, *dl*-camphorsulfonic acid, benzenesulfonic acid, and *n*-butylsulfonic acid.

The ammonium salts of these acids are soluble in acetone. On addition of dry ammonia gas, the amino acids are precipitated from such solutions when trichloroacetic acid, benzenesulfonic acid, or *dl*-camphorsulfonic acid is employed, the basic amino acids being exceptions. Arginine, histidine, and lysine separate in combination with the reagent acid. When *p*-toluenesulfonic acid, 4-nitrochlorbenzenesulfonic acid, or *d*-camphorsulfonic acid is employed as reagent, the amino acids separate in great measure in the form of complexes when dry ammonia gas is introduced in excess.

When an acid hydrolysate of casein is dissolved in acetone with the aid of trichloroacetic acid, benzenesulfonic acid, or *dl*-camphorsulfonic acid, and an excess of ammonia is introduced, about 88% by weight of the sample is recovered in the resulting precipitate. In the case of individual amino acids brought into solution in this way, recovery on precipitation is not far from 100%.

Table 1 shows the molecular ratios between reagent acid and amino acid necessary to bring the latter into solution in acetone when the concentration of acid in acetone is as indicated.

TABLE 1

Amino acid	Moles of acid reagent / Mole of amino acid		
	Trichloroacetic acid*	*dl*-Camphorsulfonic acid†	Benzenesulfonic acid‡
Proline	4.5	1.1	1.0
Threonine	5.5	1.4	1.6
Tyrosine	68.0	1.4	1.4
Isoleucine	5.1	1.0	1.6
Alanine	5.0	1.6	1.8
Valine	5.1	1.4	1.8
Aspartic acid	53.0	1.6	2.0
Phenylalanine	5.2	0.9	3.2
Serine	20.0	0.8	3.4
Hydroxyproline	25.0	2.1	3.6
Leucine	5.4	1.6	4.2
Norleucine	5.4	1.3	7.4
Tryptophan	3.0	28.6	10.6
Cystine	Insol.	10.0	13.4
Glycine	4.2	>22.5	16.2
Glutamic acid	55.0	1.7	44.0
Methionine	6.2	0.9	64.0
Histidine	6.8	3.7	128.0

* The trichloroacetic acid in acetone was a 0.24 N solution.
† The *dl*-camphorsulfonic acid in acetone was a 0.2 N solution.
‡ The benzenesulfonic acid in acetone was a 0.4 N solution.

Fractionation of hydrolysates or other mixtures of amino acids has been accomplished by the following procedures:

(1) Fractional solution: Increments of reagent (acid in acetone) are brought successively into contact with dry hydrolysate or other mixture of amino acids, in the finely ground state, contained in a filter funnel or crucible. After brief contact the reagent, containing some dissolved amino acids, is removed by applying suction. The receiver is changed, and another increment of reagent is applied to the undissolved portion of the hydrolysate, and this is then removed by suction. In this way a hydrolysate can be separated within 2 or 3 hrs into as many as 50 or 60 fractions.

(2) The hydrolysate can be completely dissolved in the minimum amount of the acid-acetone reagent, and fractions of amino acids can then be dropped out by the stepwise introduction of dry ammonia to precipitate successive fractions of the dissolved amino acids. Ammonia can be introduced in the form of a strong solution

in acetone, but this leads to a change in relation of acetone to reagent.

(3) The hydrolysate in solution may be separated into fractions by the stepwise introduction of hydrogen chloride, either directly as the gas or by means of a strong solution of hydrogen chloride in acetone.

(4) Fractionation of solutions can be brought about by successive additions to the acetone-reagent-amino acid system of suitable amounts of some solvent which is miscible with acetone but which does not have a solvent action on the amino acid-reagent complexes.

(5) Trichloroacetic acid solutions of amino acids in acetone undergo spontaneous precipitation owing to the fact that amino acids catalytically decompose trichloroacetic acid into chloroform and carbon dioxide. As the acid is decomposed, the amino acids progressively precipitate. Chromatographic analysis of fractions obtained in this way show that individual amino acids do not drop out successively but that several come out together. There is, however, in this procedure a method for obtaining greatly simplified amino acid mixtures as compared with the hydrolysate from which the fractions are obtained.

Fractions of amino acids obtained by the above-described principles from hydrolysates of proteins have been submitted to examination by paper chromatography according to the method described by Consden and his associates (*1*). The results show that from hydrolysates containing 18 or more amino acids the components of a series of 20–60 successive fractions obtained by method 1 differ in their qualitative composition. Those amino acids most readily soluble in the reagent employed tend to be most abundant in the earlier, and the least soluble ones tend to accumulate in the later, fractions.

When such fractions are rechromatographed at successive dilutions, one after another of the constituent amino acids fails to appear on the developed chromatogram. By this means it is possible to secure an approximate appraisal of the quantitative composition of any fraction with respect to its component amino acids. Each amino acid in a fraction can be identified by chromatographing the fraction in parallel with seedings of pure amino acids. The known keeps step with the unknown and reveals its identity.

Application of the available color reactions for individual amino acids to the fractions obtained by the methods described reveals that these amino acids are not present throughout the series but are found only over limited ranges of the successive fractions.

Extensive fractionation of a hydrolysate, identification and approximate quantitative analysis of successive fractions, provide information as to the similarity of fractions. This may then be used as a basis for the recombination of similar fractions in order to reduce the number of fractions to be subsequently worked with in further separation of the components. In the case of certain fractions it has been found that refractionation with the same reagent may lead to further simplification of the composition of the mixtures of amino acids which they contain. But, as will be seen from the accompanying table, advantageous procedures for isolation of individual amino acids are available by changing to a second reagent for further fractionation.

We are pursuing our investigation of the possibilities of applying the principles here described to the isolation of individual amino acids from partially and completely hydrolyzed preparations from proteins. Our studies, details of which will form the subjects of later communications, have indicated the practicability of these methods.

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The Control of Grass Weeds in Sugar-Cane Fields in Puerto Rico

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Mayaguez, Puerto Rico

In the course of experiments and field tests conducted during the last three years with herbicides for the control of weeds infesting sugar-cane lands in Puerto Rico it was soon found that the grass weed population increased when the broad-leaved plants like "cohitre" (*Commelina logicaulis* Jacq.), *Amaranthus spinosus* L., and "bejuco de puerco" (*Ipomoea* spp.) were destroyed by 2,4-D or other herbicides. The need arose for an effective grass herbicide complementary to 2,4-D or for a substitute which might control both broad-leaved and grass weeds.

It has been generally recognized that 2,4-D has little, if any, effect on grasses. Recently, Mangual (*2*) reported that the addition of 2,4-D increased the herbicidal action of oil emulsion (pentachlorophenol in diesel oil) and Concentrate 40 in grass control. Crafts (*1*) recommends an oil emulsion contact spray (pentachlorophenol in aromatic oil) for the control of young grass seedlings and claims that the addition of 2,4-D to the spray further adds to its value, providing a lethal agent for the weeds easily controlled by 2,4-D.

The results of experiments conducted on various soil types with ample replications agree with the above on the value of pentachlorophenol and the combined 2,4-D-pentachlorophenol in the control of grasses. We have further found that 2,4-D in oil provides a more effective grass herbicide than pentachlorophenol emulsion or Concentrate 40.

In a typical experiment on fallow ground and repeated three times, plots were treated with an aqueous solution of 0.1% 2.4-D to kill most of the broad-leaved plants, allowing the grass weeds to develop without competition. The most prevalent volunteer weeds on the plots were *Cyperus rotundus* L., Bermuda grass (*Cynodon Dactylon* (L.) Pers.) *Eleusine indica* (L.) Gaertn., *Digitaria sanguinalis* (L.) Scop., and *Eriochloa polystachya* H.B.K. Sixteen plots were measured out, 20′ × 8′, and a 4′ section of each was hoed, spaded, and planted to *Trichachne insularis* (L.) Nees, a grass resistant to most chemical herbicides. Four stools of sugar cane were planted in each plot to determine susceptibility or resistance to

chemical treatment. Three plots were thoroughly sprayed with each of four chemical treatments, three plots were flamed with a flame thrower at intervals of three weeks, and one remained untreated.

The results were recorded at the end of 14 weeks on what seemed to us to be a fair approach to a quantitative basis. In Table 1 effectiveness of treatment is given in percentage of destruction of weeds. The percentage of plants remaining alive or arising from unaffected root-stocks or seeds was taken in relation to the number of living plants in the check plot.

TABLE 1

Weed	Percentage destruction by treatment*				
	A	B	C	D	E
Eleusine indica	100	85	98	100	100
Cynodon Dactylon	95	80	90	80	50
Digitaria sanguinalis	100	80	90	85	50
Eriochloa polystachya	90	25	80	80	0
Trichachne insularis	90	50	90	50	100
Cyperus rotundus	90	25	80	90	0

*A = 150 ml of 40% 2,4-D ester/gal of diesel oil; B = 1 lb of pentachlorophenol/15 gals of diesel oil; C = 1 lb of pentachlorophenol, 1 liter of 40% 2,4-D ester, 15 gals of diesel oil; D = Dow G-502, dinitro-*o*-secondary butyl phenol, 1 gal, 1 liter of 40% 2,4-D ester, 10 gals of diesel oil; E = firing.

Effective control was obtained with 2,4-D (150 ml of 40% ester/gal of diesel oil) at the rate of 40–50 gals/acre. Two other treatments applied at the same rate per acre were very effective: (a) dinitro-*o*-secondary butyl phenol (Commercial Dow Contact) in combination with 2,4-D and diesel oil (1 gal of Dow Contact, 500 ml of 40% 2,4-D ester, and 10 gals of diesel oil) and (b) pentachlorophenol with 2,4-D and diesel oil (1 lb of pentachlorophenol, 1 liter of 40% 2,4-D ester, and 15 gals of oil). Firing to destroy all above-ground parts of a grass weed stand proved to be ineffective. *Cyperus rotundus* was rather stimulated in growth. Likewise, there was stimulation in the germination of seeds of *Amaranthus spinosus* L. and other plants.

All the treatments were highly injurious to cane when the spray was applied to plants younger than 4 months of age. Older plants escaped injury when the spray was applied carefully.

More recent experiments have shown that aromatic oils (Shell) are far more effective than diesel oil, making it possible to reduce the quantity of 2,4-D ester to 75 ml/gal of oil.

It is evident that 2,4-D, which alone has little effect on grasses, becomes a very active grass herbicide when dissolved in diesel or aromatic oil and applied as a fine spray. The practical implications will be clear when one considers that grass weeds have become the most pernicious competitors of sugar cane for soil nutrients and soil moisture.

It is recognized that such an active herbicide has to be applied with extraordinary care to growing cane. If the 2,4-D oil spray is applied on a windy day, much injury may result to leaves of cane. To obviate these shortcomings of the treatment, two alternative methods of application have been developed. The first involves a preplanting application. Fields are prepared in the ordinary way, including furrowing and ditching, and irrigation water is then applied. Grass weeds are allowed to grow, and at the end of 3 weeks the 2,4-D oil spray is applied either with knapsack or power sprayers. Cane is planted the day following the herbicide application. This will considerably reduce the grass growth while the young cane is growing. Hoeing or other forms of mechanical cultivation are not eliminated altogether, but much labor saving is effected. The second method requires that the 2,4-D oil spray be applied to fields of growing cane when this is over 3 months of age. A combination of the two methods has been found to reduce effectively the number of hoeings from 6 or 7 to 3 in new plantings.

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Demonstration of Reducing Enzyme Systems in Neoplasms and Living Mammalian Tissues by Triphenyltetrazolium Chloride

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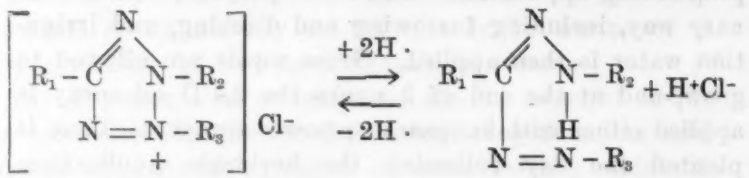
Tetrazolium salts first prepared by Pechman and Runge (*6*) in 1894 and extensively investigated by Kuhn and Jerchel (*3*) were proposed by Lakon (*4*) for testing the viability of seeds. Attention to the usefulness of these reagents was pointed out in this country by the report of Dutcher (*2*), who interrogated Lakon in 1945. Subsequently, Porter, Durrell, and Romm (*7*), Mattson, Jensen, and Dutcher (*5*), Waugh (*9*) and Cottrell (*1*), confirmed the observations of Lakon and indicated that tetrazolium salts may be useful as reagents to detect differences in the viability of seeds and other tissues.

We synthesized a series of tetrazolium salts including the 2,3,5-triphenyl and 2,3-diphenyl-5-methyl compounds. The present paper deals with the demonstration of reducing enzyme systems in neoplasms and living mammalian tissues by means of the triphenyltetrazolium salt.

Warburg and Christian (*8*), in 1943, showed that the plasma of animals bearing large sarcomata contained an increased quantity of zymohexase and postulated that, in contradistinction to normal mammalian cells, the tumor cell obtains energy from glycolysis even in the presence of available oxygen. It appeared to us that, if there were an appreciable difference in the amount of glycolysis in the neoplastic cells as compared to normal cells in contact with available oxygen, such a difference might be demonstrated by reagents capable of reacting with enzymes in the chain of glycolytic fermentation. Tetrazolium salts

are water-soluble, colorless substances; their solutions, in contact with certain reductases in living tissues, are reduced to insoluble, red substances called formazans:



Colorless Tetrazolium Salt — Colored Formazan

It is probable that all living cells contain enzymes of the glycolytic system. The concentration of the enzyme varies with the type of cell and the individual characteristics of that cell's demand for energy, and the availability of oxygen for alternative cytochrome oxidation. It is believed that normal mammalian cells will utilize the more efficient cytochrome oxidation preferentially as a source of energy, when the oxygen supply is adequate; further, it is reasonable to assume that a neoplastic cell which obtains energy from glycolysis may reduce tetrazolium salts at a faster rate than normal cells. This possibility may furnish a tool useful in the study of cancer cell metabolism and cellular anoxia in general.

Triphenyltetrazolium chloride, injected intramuscularly in rabbits, causes no apparent injury in doses of 100 mg/kg, using a 2% solution. Injection of 150 mg/kg, using a 10% solution, is lethal within 25–30 min. The serum of a rabbit which received a lethal dose of the salt was colored ruby red by the formazan. Decoloration of the serum and reduction by means of sodium stannite demonstrated the presence of still unreduced tetrazolium salt.

Intravenous injections are more toxic than intramuscular injections. A dose of 25 mg/kg in the form of a 1% solution can be tolerated with no apparent injury. Examination of the anaesthetized animal after 90 min and 24 hrs did not disclose any pigmentation of the tissues. However, intravenous injections of 100 mg/kg, in the form of either a 5% or a 10% solution, resulted in immediate collapse. The heart contracted for 5 min after cessation of respiration and then stopped. At this time inspection of the viscera showed no color. Within 10 min the upper small intestine became rose colored. In the next 10 min most of the small bowel, the visible muscles, and heart became pink-red. Later, the skeletal muscles became rose-red, and the upper small bowel became scarlet. At this time the lower small bowel and colon were rose-red, while the liver, spleen, kidneys, and adrenals were not colored. It may be significant that reduction occurs in the vicinity of still living or very recently living cells after cessation of circulation has reduced the oxygen available to the cells.

We have found that there is a differential reduction of the tetrazolium by carcinomatous tissue as compared to the rate exhibited by the surrounding tissues. Segments of freshly removed carcinomatous tissue immersed in 1% tetrazolium chloride at room temperature or at 37° C develop a faint pink color within a few minutes and become ruby-red within 20 min. The uninvolved surrounding tissues remain unstained. Microscopic examination of tumor sections immersed in tetrazolium chloride shows a diffuse reddish pigment distributed both within the epithelial cells and in the stroma. It has been found that the carcinoma can be frozen upon removal, then sectioned and stained by incubation in tetrazolium chloride solution for 20 min. Mammary carcinoma tissue, allowed to stand for 3 hrs at room temperature (in a moist environment), then immersed in the tetrazolium chloride solution, develops a much fainter color than when utilized immediately after amputation. Freezing immediately after amputation is indicated as a means of preserving the enzyme system which reduces the tetrazolium salt. Heating the carcinoma tissue at 100° C for 5 min entirely inhibits the development of color. Table 1 gives a summary of the staining of human tissues by tetrazolium chloride.

TABLE 1

Tissue (human)	Region	Temp. (°C)	Time	Staining
		(Immersion in 1% tetrazolium chloride sol.)		
1. Carcinoma	Breast	Room	20 min	Deep red
2. "	"	37	20 "	Deep red
3. Uninvolved breast	" #2	37	20 "	Light pink
4. " skin	" #2	37	20 "	Basal layer, fine red line (remainder unstained)
5. Carcinoma	"	37	3 hrs	Dark red
6. "	Pancreas	37	15 min	Red-brown
7. Fibroadenoma	Breast	37	3 hrs	No color change
8. Normal jejunum	Gastrojejunostomy	Room	15 min	" " "
9. " "	"	37	3 hrs	Red
10. " skin	Abdomen	37	1 hr	No color change
11. " "	"	37	24 hrs	" " "
12. Duct papilloma (malignant?)	Breast	37	3 "	Ducts faint pink, tumor bright red
13. Uninvolved breast	" #12	37	3 "	No color change
14. Mixed tumor parotid (malignant?)	Neck	37	15 min	" " "
15. Mixed tumor parotid	Case #14	37	2 hrs	" " "

It is of interest to note that tissues from a noncarcinomatous area in a carcinomatous breast were faintly stained, and that uninvolved skin from the same breast showed some reduction, while fibroadenomatous breast and skin from an appendectomy incision showed no color.

The application of the tetrazolium salts as intravital dyes is further suggested by the following observations. A gauze pack saturated with 1% tetrazolium chloride was applied to an ulcer surface which developed after postoperative irradiation of a carcinoma of the anus. It was not possible to determine by inspection whether the ulcer was due to recurrence of the carcinoma or to a late radiation necrosis. After 12 min the ulcer surface was stained bright scarlet-red, as was the gauze surface in contact with it. Removal of the ulcer and surrounding tissue revealed that the staining was limited to the surface of the ulcer to a depth of 2 mm below the surface. Frozen sections showed the presence of squamous cell carcinoma. A similar observation was made on an ulcer of the tongue. A cotton applicator saturated with 1% tetrazolium chloride was held on the ulcer for 10 min. The ulcer surface and the applicator were stained scarlet. Excision of the tongue showed the lesion to be a squamous cell carcinoma. Granulation tissue covering a third-degree burn ulcer was treated similarly for 20 min, but no color developed.

There is a suggestion that the tetrazolium compound is reduced more rapidly than by normal tissues, in regions where a local deficiency of oxygen exists. A leg was amputated for arteriosclerotic ischemia. Skin, deep fascia, and gastrocnemius muscle were taken from the fresh leg at the level of the knee joint and immersed in 1% tetrazolium chloride solution for 20 min at 37° C. No color developed. Similar skin, deep fascia, and muscle were taken from just above the internal malleolus of the tibia, 2″ above a gangrenous ulcer, and also immersed and incubated. The muscle and fascia showed no color although the skin was colored a faint pink, visible through the epidermis. On section it was demonstrated that the superficial layers of the epidermis were not colored. The cutis vera was a moderate pink color, and a fine scarlet line outlined the probable course of the basal layer of the epidermis.

These observations indicate that tetrazolium compounds may be used as a tool in the study of the intracellular metabolism of tissue anoxia. The scarlet-red color which develops on reduction is not ideal, since some mammalian tissues are only a different red. Work is under way not only to synthesize tetrazolium salts which may give formazans of other colors, preferably blue or green, but also to determine the fate of the tetrazolium chloride and to study the enzyme system which affects the rate of reduction in the organism. It is possible that a soluble substance which can be precipitated in differential quantities in some neoplastic tissues may prove a useful agent in the study and treatment of neoplasms.

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Palynological Studies at Sodon Lake: I. Size-Frequency Study of Fossil Spruce Pollen[1]

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In another paper of this series Cain and Slater (*3*) are reporting a pollen analytical study of a 24′ profile of the peat and marl sediments of Sodon Lake; and Cain and Cain (*2*) have made a size-frequency study of the fossil pollen grains at Sodon Lake in relation to the modern species, showing something of the historical successional relationships of *Pinus Banksiana, P. resinosa,* and *P. Strobus.* The present paper considers the question of whether it is possible to identify the species of *Picea* which contributed to the fossil pollen of the blue clay layer of the 24′ level at the bottom of the profile.

The vegetational history of Sodon Lake, Oakland County, Michigan, in so far as it is revealed by the stratigraphic column, commences with the pre-Boreal spruce-dominated pollen spectrum referable to Period I in the scheme of Sears (*7*). This is the only level at which spruce is dominant. Of the 322 grains of this level, 85.7% were recognized as spruce, and about 100 of these were in a sufficiently good state of preservation and were oriented properly for measurement of the grain size. Grains seen squarely at right angles to their long axis (in dorsal, ventral, or lateral view) were measured across the maximum dimension of the grain, within the exine. This position is often along a line connecting the points of dorsal insertion of the bladders. The grains were found to range between 61.6 and 97.6 μ. The number of measurable grains was insufficient to produce a smooth size-frequency curve (Fig. 1, heavy-line curve) or to indicate surely whether one or more species of *Picea* were involved in the sedimentation.

[1] In the "Pollen Analysis Circular" Dr. Antevs posed the question: "Is pollen analysis the proper name for the study of pollen and its applications?" In No. 8 of the circular (page 6, October 1944) H. A. Hyde and D. A. Williams, of Wales, suggested the term *palynology* (from Greek *paluno,* to strew or sprinkle; cf. *pale,* fine meal, cognate with Latin *pollen,* flour, dust): the study of pollen and other spores and their dispersal, and applications thereof. Erdtman (1947) has accepted the term. Since it seems appropriate, we are using it formally for a series of papers somewhat broader in scope than those formerly included under "pollen analysis."

The next step was to prepare pollen from available contemporaneous material of the black spruce (*Picea mariana*) and the white spruce (*P. canadensis* or *P. glauca*) and to establish size-frequency curves and suitable statistics for the species. These species were selected because they are the ones present in Michigan today (see the map, Fig. 2). Fig. 1 shows size-frequency curves obtained for black spruce pollen (herbarium specimen from Isle Royale, Michigan) and for white spruce (herbarium specimen from Charlevoix, Michigan). The results were disappointing, for the black spruce, which occurs even today on bogs within Oakland County, Michigan, within a few miles of Sodon Lake, is obviously much smaller than most of the fossil grains, its curve overlapping the size-frequency distribution for fossil grains only slightly. The larger pollen grains of the white spruce essentially cover the whole size range of the fossil grains, but the modal size of the distribution suggests that this species probably did not contribute more than half of the fossil grains.

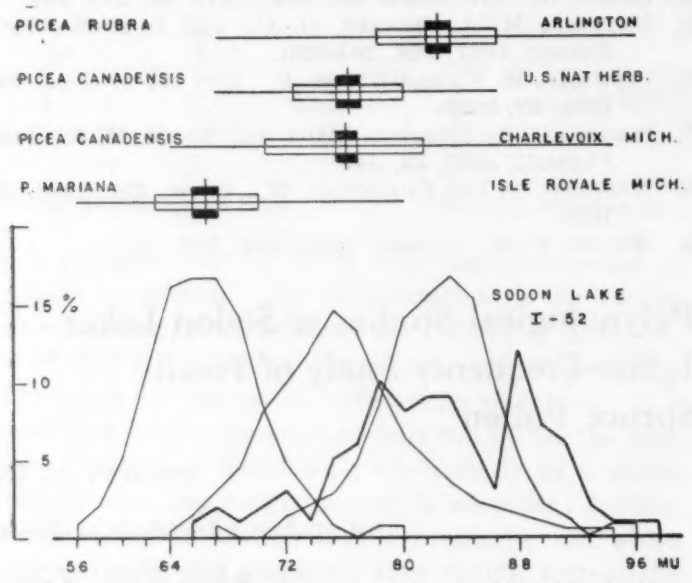

FIG. 1. Size-frequency and other statistical data for pollen grains of three species of spruce, and for fossil grains at Sodon Lake.

FIG. 2. Approximate areas of *Picea mariana* (Mill.) B.S.P., *P. glauca* (Moench) Voss, and *P. rubra* Link.

After speculating about this difficulty, the writer decided to compare the size-frequency characteristics of the eastern red spruce (*P. rubra*) with the fossil grains.

TABLE 1

SIZE-FREQUENCY CHARACTERISTICS OF *Picea* POLLEN

Size class (ocular lines; 1 line = 1.6 μ)	*P. mariana* Isle Royale, Michigan	*P. canadensis* (*glauca*) Pollen from U.S.N.H.	*P. canadensis* (*glauca*) Charlevoix County, Michigan	*P. rubra* Arlington Chemical Co.	Fossil *Picea* pollen Sodon Lake
36	0.7				
37	2.1				
38	5.3				
39	10.7				
40	16.1		0.5		
41	16.6		1.5		1
42	16.6	1.3	3.0	0.7	2
43	14.0	2.6	4.5	..	1
44	8.6	4.7	8.0	..	2
45	4.7	9.3	10.0	1.3	3
46	2.6	13.3	12.0	2.1	1
47	0.7	18.0	14.5	2.6	5
48	..	17.3	13.0	4.7	6
49	0.7	12.0	9.5	8.6	10
50	0.7	8.6	6.5	14.0	8
51		6.0	5.0	15.4	9
52		4.7	3.5	16.6	9
53		1.3	2.5	15.4	6
54		0.7	2.0	9.3	3
55			1.5	4.7	12
56			1.0	2.1	7
57			0.5	1.3	6
58			0.5	..	2
59			0.5	0.7	1
60				0.7	1
61					1
N	150	150	200	150	96
$\overline{X}$	66.86 μ	76.70 μ	76.54 μ	82.80 μ	..
σ	3.86 μ	3.82 μ	5.43 μ	4.25 μ	..
PE$\overline{x}$	0.21 μ	0.21 μ	0.26 μ	0.23 μ	..
$2\frac{\sigma}{\sqrt{N}}$	1.01 μ	1.00 μ	1.22 μ	1.12 μ	..

Students of fossil pollen in the western Great Lakes area have never considered the possible presence of more than the two presently native species of the area. Most students have only designated the genus, but Wilson (*8*), Wilson and Kosanke (*9*), Wilson and Webster (*10*), and Friesner and Potzger (*5*) have named *Picea mariana* and *P. glauca* from sediments they have studied, without, however, publishing adequate statistical or other diagnostic characters. Not having studied these characteristics of the red spruce before, the writer was both surprised and pleased to find that at least the one collection of pollen available showed the species to have grains

sufficiently large to account for the balance of the size-frequency array for fossil grains at Sodon Lake.

To Fig. 1 has been added a very useful type of graphic presentation of statistical data originated by Dice and Leraas (*4*). The data forming the basis for the diagrams are presented in Table 1. The horizontal line marks the range for each population. The transverse or vertical line marks the position of the mean ($\overline{X}$). The open rectangle marks off one standard deviation (σ) on each side of the mean; and the black, superimposed rectangle marks off 2 standard errors ($2\frac{\sigma}{\sqrt{N}}$) each side of the mean. It is thus apparent that there is no significant statistical difference between the two samples of pollen of the white spruce. It is equally obvious that the three species (in so far as they are represented by the presently available material) have pollen grains of such size that adequate samples easily permit their statistical separation, despite the considerable overlap of ranges.

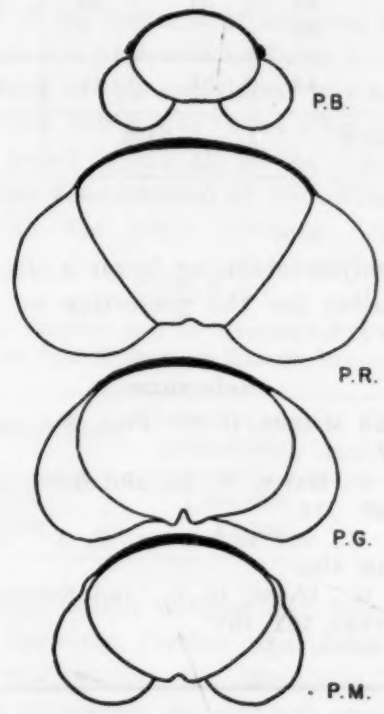

FIG. 3. Median optical views of pollen grains of the $\overline{X}$ size class for each species. (P. B.) *Pinus Banksiana* (45 μ), added for comparison with the spruces, (P. R.) *Picea rubra* (83 μ), (P. G.) *P. glauca* (77 μ), (P. M.) *P. mariana* (67 μ).

Despite frequent confusion in the identification of the eastern American spruces, and the fact that the red spruce has sometimes been attributed to the western Great Lakes area in its modern distribution, there seems to be no doubt that today the species is nearly strictly Appalachian and New England in occurrence (Fig. 2), based on Munns (*6*). We face the question, then, whether the present data are sufficient to warrant the conclusion that the red spruce was present far west of its modern area during pre-Boreal time; specifically whether it was in the neighborhood of Sodon Lake, in southeastern Michigan. The hypothesis that it was is a reasonable one. It is also apparent, however, (*1, 2*) that different collections of pollen of the same species may have statistically significant size differences; hence, there is an urgent need for further size-frequency studies of these species. Verification of the size-frequency characteristics of these three species of *Picea* and further measurements of fossil grains in other profiles should strengthen the circumstantial case for the former extension of range of the red spruce.

Further evidence for the presence of fossil red spruce pollen grains in the Sodon Lake sediments lies in the forms of the grains, many of which match perfectly the typical form of this species (Fig. 3, P.R.). Pollen grains are somewhat variable in form, but the illustrations show scale drawings of grains that are typical both as to size and shape. It is not claimed that individual pollen grains of spruce can be identified and that percentage composition of the species can be determined for use in pollen spectra, but it does seem that by a combination of size and form characteristics, when numerous grains are available from a particular sediment, the presence of certain species can be detected.

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Inhibition of Glycolysis *in Vitro* by Impure Penicillin[1]

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It has been reported by Miller, Hawk, and Boor (*2*) that impure penicillin protects mice against bacterial endotoxins. Since it was found that bacterial endotoxins cause marked disturbances in the intermediate metabolism (*1*), it seemed of interest to search for metabolic effects of impure penicillin in the hope of gaining an insight into the mechanism responsible for its protective action.

Mouse liver and muscle and mouse sarcoma 37 were homogenized in ice-cold distilled water by means of glass homogenizers (*3*), and glycolysis was determined manometrically by the system of Utter, Wood, and Reiner (*4*). Each Warburg flask contained 20 mg of tissue homogenate. Glycolysis was expressed in terms of mm^3 of CO_2 formed in 20 min, after the homogenate was mixed with the contents of the main compartment. Impure penicillin[2]

[1] This investigation was supported by the U. S. Navy, Office of Naval Research, and the University of Chicago.

[2] An intermediate product in the commercial production of penicillin, kindly supplied by the Abbott Laboratories.

containing approximately 5,000 units of penicillin/ml (4.1% content of solids including phosphate buffer) was added in 0.5-ml volume/flask.

fect on glycolysis, indicating that the inhibition of glycolysis is due to some other factor present in the impure preparation. It cannot yet be stated whether

TABLE 1

INHIBITION OF GLYCOLYSIS OF MOUSE TISSUES BY IMPURE PENICILLIN

No. of experiment	Liver				Muscle				Sarcoma			
	Aerobic		Anaerobic		Aerobic		Anaerobic		Aerobic		Anaerobic	
	C*	IP*	C	IP	C	IP	C	IP	C	IP	C	IP
1	58	30	64	37	68	37	75	39	59	41	29	16
2	61	32	69	39	71	40	88	46	60	45	29	16
3	57	29	64	31	66	30	79	40	56	43	26	14
4	59	31	66	35	65	33	86	44	57	46	29	14
5	63	30	70	40	69	41	82	45				
Average	60	30	66.7	36	68	36	82	43	58	44	28	15
Difference	50%		46%		47%		48%		24%		47%	

* C = control; IP = impure penicillin added.

Results are given in Table 1. These show that both aerobic and anaerobic glycolysis of liver and muscle homogenates was equally inhibited, suggesting that the site of action of the impure penicillin is in the anaerobic part of the glycolytic cycle. The sarcoma gave a higher aerobic than anerobic glycolysis, which is typical for malignant tissues. The impure penicillin caused less inhibition of the aerobic glycolysis of the sarcoma, but inhibited its anaerobic glycolysis to the same degree as in normal tissues.

Crystalline penicillin G (15,000 units/ml) had no effect on glycolysis, indicating that the inhibition of glycolysis is due to some other factor present in the impure preparation. It cannot yet be stated whether or not the glycolysis-inhibiting factor is identical with the factor responsible for the protection of mice against endotoxins.

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IN THE LABORATORY

Benzene-Ether Extracted Rabies Vaccine

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In 1946–47 DeBoer and Cox (*1*) described a method for the preparation of complement-fixing antigens from brain by benzene (C_6H_6) extraction. Espana and Hammon (*2*) confirmed the validity of the principle and slightly modified the method of extraction. It occurred to us that the procedure might be adapted to the removal of lipid from brain tissue, as an initial step in the purification of rabies vaccine. Previously we had attempted by various common procedures to separate rabies virus from tissue elements. In some instances it seemed that the lipids of the brain may have interfered with sharp separation. In view of the results obtained by DeBoer and Cox, we attempted the preparation of vaccines by extracting lipids with various solvents.

Earlier experiences with the exposure of rabies virus to ether revealed that the virus is quite labile to ether when wet. Consequently, the first step was drying of the homogenized, infected, brain tissue from a frozen state under vacuum. Various lipid solvents were then added to the dry tissue, the volumes in each case being about twice the wet volume of the original tissue suspension. After a period of about 2 hrs in the cold room, the solvent was removed either by centrifugation and decanting or by filtration through a sintered glass filter of "C" porosity. In either case the sediment (or residue) was resuspended in the solvent and immediately recentrifuged or refiltered. Following the extration with benzene or other solvent, ether [$(C_2H_5)_2O$] extraction was done twice in a similar way but using ether at $-50°$ C. The tissue was then freed of the small amount of residual ether by placing the container in a jar and exhausting by water suction. Rehydration was accomplished simply by shaking with water and churning in a Waring blendor. The tissue suspensions were stored

in the refrigerator at +5° C, and samples were removed at intervals for the vaccination of mice, utilizing the technique of Habel and Wright (*4*).

TABLE 1

POTENCIES OF ANTIGENS EXTRACTED WITH VARIOUS SOLVENTS

Solvent used	Viability at 10^{-3} dil.	Antigen LD_{50} protection
Acetone	–	15,000
Benzene	+	290,000
Carbon tetrachloride	–	100
Dichlorethylene	–	3,000

The results of the first test of antigens extracted with various solvents are presented in Table 1.

The virus used in this experiment was not inactivated before extraction, and it was found after extraction that benzene had failed to kill all of the rabies virus. No viable virus was demonstrated at 10^{-3} dilution following extraction with the other solvents. Since benzene yielded the most potent antigen and was found to be least toxic to the virus, further experiments were undertaken using this solvent for extraction. It was determined that extraction as outlined above effected a marked reduction of the lipid content of the dry vaccine. The effect of cold benzene and ether on the titers of virus in two different lots is presented in Table 2.

TABLE 2

THE EFFECT OF DRYING AND OF BENZENE-ETHER EXTRACTION ON THE TITERS OF RABIES VIRUS

	Original infective titer	Infective titer after drying	Infective titer after extraction
1)	10,000,000	1,000,000	1,000
2)	501,000	13,000	400

Since extractions with benzene and ether in the cold did not completely inactivate rabies virus, subsequent extractions were done on mouse brain suspensions previously inactivated by ultraviolet light (*3, 5*). In each case another sample of the same irradiated lot, dried but not extracted, was used as a control. In two experiments the dried, or dried and extracted, tissue was treated with phenol to kill the virus. The relative potencies of extracted and unextracted vaccines are compared in Table 3.

TABLE 3

COMPARISON OF THE POTENCIES OF EXTRACTED AND UNEXTRACTED, NONVIABLE VACCINES

Experiment No.	LD_{50} protection by control vaccines (unextracted)	LD_{50} protection by extracted vaccines
1*	0	36,000
2	10,000	274,000
3	10,000	274,000
4	568,000	568,000
5	4,000	400
6†	700	9,000
7†	100	9,000

* The source material was an outdated vaccine.
† Phenol killed.

It is evident that the antigenicity of extracted vaccines is as good as, or better than, that of unextracted material of the same lot in most instances. No explanation is offered for the observation, but the experiments listed, and others to be reported later, consistently suggest significant difference in favor of the extracted vaccines. In our hands, experimental phenolized vaccines have tended to be of lower antigenicity than corresponding irradiated vaccines.

It has thus been found that extraction of rabies vaccine with cold benzene followed by cold ether will remove much of the lipid (41.5% of the total dry weight) of vaccine. The process causes a marked reduction in the infective titer of rabies virus in brain tissue, but does not kill all of the virus under the conditions described. Rabies vaccines killed with ultraviolet light and extracted do not lose antigenicity, but in most instances are actually improved as antigens. As a result of our experiments we have concluded that benzene and ether-extracted vaccines may offer some advantage over unextracted vaccine for the prevention of rabies. However, the greatest advantage of lipid extraction is the elimination of one of the major nonspecific components of a very crude vaccine.

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A Constant-Temperature Micromanipulation Chamber

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Although the constant-temperature micromanipulation chamber to be described here was developed in connection with certain studies in progress on mammalian tissue, it, unlike the special constant-temperature apparatus described by Peterfi (*3*), has a wide range of application not only in this general field of investigation but also in other fields not employing the micromanipulative technique. This instrument was designed specifically for use with the Chambers micromanipulator (*1*), but its basic

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functional features may be adapted to those types of chambers required by other manipulators (*1*).

The instrument is constructed entirely of sheet Plexiglas, and the parts are welded together with Plexiglas solvent. The thermal, electrical, optical, and other properties of Plexiglas make it a material of choice. The apparatus consists essentially of two units: a manipulation unit (Fig. 1A) and a gas-mercury thermostat (B). The manipulation unit has an outside measurement of $2'' \times 3'' \times \frac{1}{2}''$ with an off-center moist chamber (C) of essentially standard dimensions (*2, 4*). It consists of four welded, appropriately shaped plates: a floor plate $\frac{1}{16}''$ thick (D), a shell plate $\frac{1}{4}''$ thick (E), a step plate $\frac{1}{16}''$ thick (F), and a roof plate $\frac{1}{16}''$ thick (G). An inner chamber (H) bounds the moist chamber (C) on three sides. In operation, the inner chamber is filled with tap water. This chamber opens to the outside through an outlet nipple (I) to which is attached a small rubber overflow tube (J). The latter is directed toward the rear edge (K) of the unit and inserted into a tube support (L). Two threaded brass electrodes (M, N) extend for a short distance into the inner chamber through the left edge (O) of the unit. Each of these electrodes is sealed watertight by means of a rubber washer (P) under compression by a plastic nut (Q). A hole (R) in the left edge of the unit accommodating the activating chamber (S) of the thermostat is made watertight by the vertical block of the thermostat (T), which is welded directly to the edge of the unit.

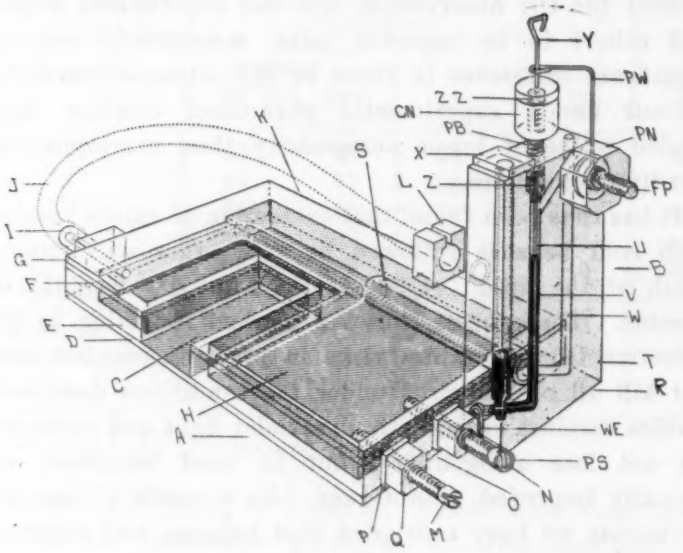

FIG. 1

The bulk of the thermostat consists of a vertical block (T) in which three vertical channels, appropriately interconnected, have been drilled. These channels together constitute an upright modified U-tube (U) partially filled with mercury (V) and a long side arm (W) connecting it to an activating chamber (S) filled with air and lying within the water filled inner chamber of the manipulation unit. The front arm of the U-tube is sealed airtight by a small plate (X). An adjustable platinum wire electrode (Y) for temperature regulation dips into the mercury column in the rear arm of the U-tube. It is sealed airtight by a rubber disc (Z) put under compression by a plastic bolt (PB) screwed into the upper end of this arm, which has been appropriately countersunk and threaded. In addition, it is sealed by means of a rubber disc (ZZ) compressed against the end of the plastic bolt by means of a plastic capping nut (CN). A fixed post (FP) for tying into the electric source consists of a brass bolt held by a recessed plastic nut (PN) welded to the side of the vertical block. A platinum wire (PW) connects this fixed post to the adjustable platinum wire electrode (Y). Another platinum wire electrode (WE), welded in position permanently, connects the mercury column in the U-tube with the rear electrode (N) of the manipulation unit. The exposed outer end of this latter electrode is insulated by means of a rubber or plastic sleeve (PS), and the wire is coated with shellac. Lead-in wires from a standard 110-volt a–c source are attached by means of insulated sleeve type connectors to the fixed post of the thermostat (FP) and to the front electrode of the manipulation unit (M).

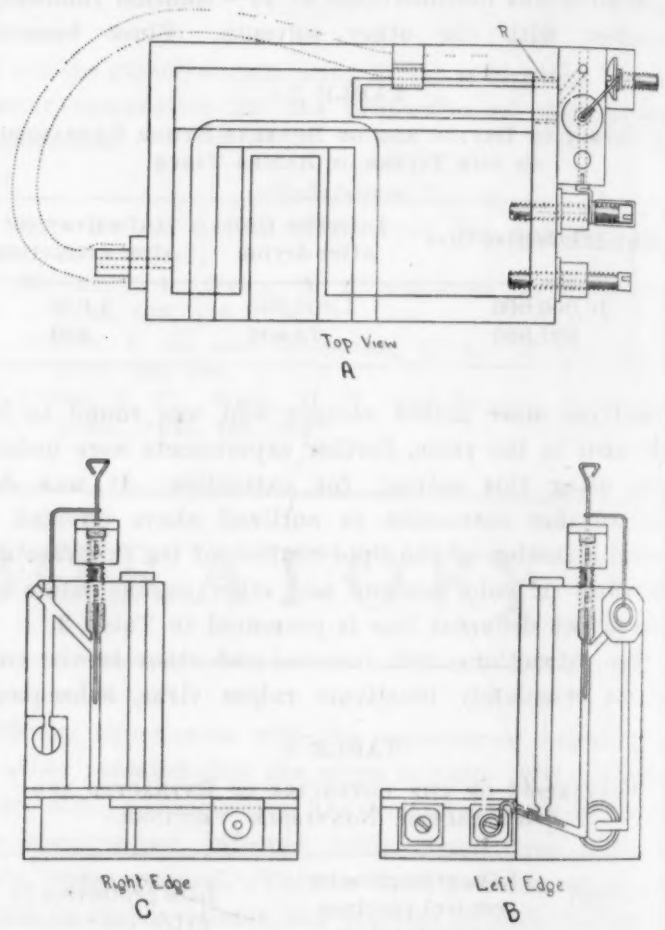

FIG. 2

The heating device consists simply of the passage of the electric current through the tap water filling the inner chamber by means of the manipulation unit electrodes (M, N). The heat generated is due to the electrical resistance of the tap water. The circulation of the water within the inner chamber depends on the formation of and the guidance of the convection currents established. The roof of the inner chamber is not of uniform height. Because of the step plate (Fig. 1F; Fig. 2A, B, C) it is low near the electrodes and higher near the far sides of the moist chamber. Consequently, as the water is heated, the warmer, and thus lighter, strata are guided almost directly into the far side of the inner chamber, making for

a quick and more even heating of the three walls of the moist chamber. Although slight, the expansion of the water within the inner chamber and the escape of small gas bubbles, which are very gradually formed, into the overflow tube probably aid also in this regard. Controlled temperatures ranging from a room temperature of 18° C to 48° C can be obtained within the moist chamber. The variation from a given temperature is, at the most, about ± .3° C.

The thermostat is operated by the expansion and shrinkage of air in the sealed activating chamber against the column of mercury in which the adjustable platinum wire electrode dips. The dimensions of the activating chamber, the connecting side arm, and the rear arm of the U-tube are such that the mercury column is moved approximately 1 mm for each °C change in temperature. When the air in the activating chamber expands, it simply pushes the meniscus of the mercury column toward, and finally away from, the end of the adjustable electrode. This breaks the electrical circuit and interrupts the heating device. When the air in the activating chamber shrinks due to cooling, the mercury column rises into contact with the tip of the adjustable electrode to complete the circuit and to activate the heating device. The thermostat is set by lowering the adjustable electrode to increase the temperature and by raising it to decrease the temperature.

This instrument is accommodated by any standard microscope and is used with the Chambers micromanipulation assembly in all respects just as if it were a standard moist chamber slide (*2*, *4*).

Detailed measurements necessary for the construction of the constant-temperature micromanipulation chamber may be obtained by applying the 1″ scale in Fig. 3 to the construction plans (Figs. 2A, B, C; Fig. 3). The mercury

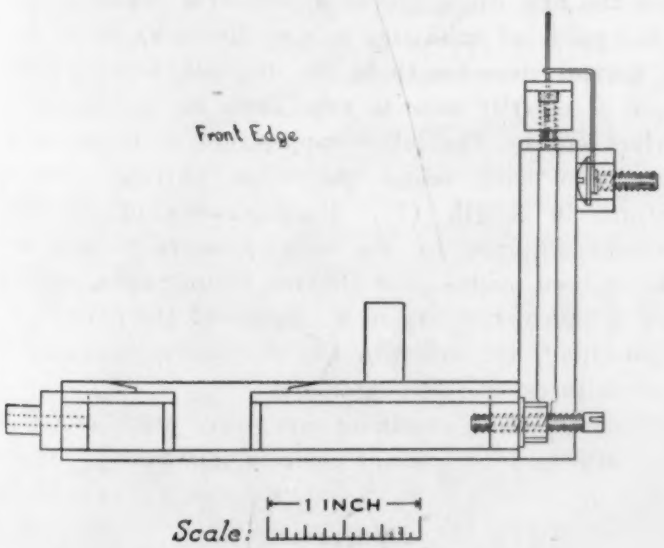

FIG. 3

is placed in the modified U-tube in two steps: (1) With the seal plate (Fig. 1X) off, mercury is added to the U-tube to the general height shown in the front arm of the U-tube in Fig. 1. (2) The seal plate (Fig. 1X) is welded into position, additional mercury is added to the rear arm of the U-tube to a point about 10 mm below the junction of the side arm, and this arm of the U-tube is then sealed with the appropriate assembly (Fig. 1).

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The Use of Thionyl Chloride in the Preparation of Schiff's Reagent

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While studies on the specificity of the Feulgen reaction were being conducted, methods for the preparation of Schiff's reagent were evaluated. Since sulfur dioxide is the agent active in the conversion of basic fuchsin to its leuco form, it does not appear to matter what source for this compound is used so long as secondary products are not produced which could affect the reaction between the dye and the sulfurous acid. Sulfite, bisulfite, and metabisulfite (*2*), as well as sulfur dioxide (*3*), have been used as sources of the sulfurous acid.

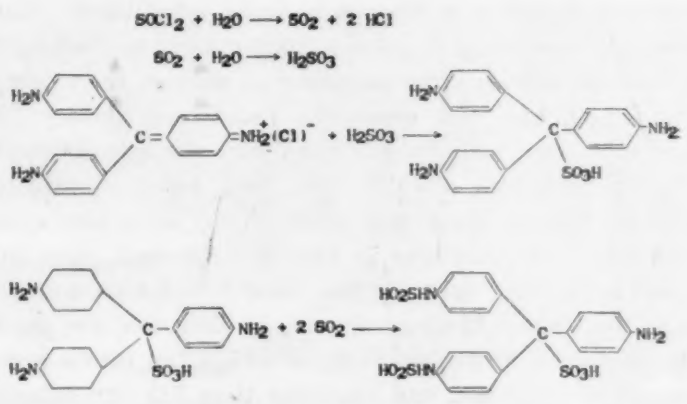

FIG. 1. Reactions which can be expected to occur when thionyl chloride is used as a source of sulfurous acid in Schiff's reagent.

The following method, in which thionyl chloride is used as the source of sulfurous acid, has been found to be both simple and effective. First, 1.0 gm of basic fuchsin is dissolved in 400 cc of distilled water. Second, 1.0 cc of $SOCl_2$ (thionyl chloride, CP) is added. The flask is stoppered and allowed to stand for 12 hrs. Third, after decolorization of the dye, the solution is cleared by the addition of 2 gm of activated charcoal, after which the mixture is shaken and immediately filtered (*1*). The solution is stored in a well-stoppered bottle.

It is likewise possible to treat the 0.25% basic fuchsin solution with charcoal prior to its decolorization and to obtain a clear, colorless solution on the addition of thionyl chloride.

In accordance with the work of Wieland and Scheuing, the reactions which occur should be as represented in Fig. 1.

The observed pH of Schiff's reagent prepared with thionyl chloride is 1.24; that of Schiff's reagent prepared in the usual manner is 1.38. This slight difference in acidity does not appear to be significant.

Schiff's reagent prepared with thionyl chloride has been successfully used as a nuclear stain on tissue sections of human thymus gland, kidney, liver, and spleen, as well as on the fungi, *Blastomyces dermatitidis* and *Saccharomyces cervisiae.*

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Plant Virus for Electron Microscopy

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The uniformity of size of an infectious unit has a significant bearing on the purity of a culture when the causative agent of a disease is to be established. Considerable variability in the size, particularly in the length, of tobacco mosaic virus particles, as seen in the electron microscope, has been reported. Oster and Stanley (*5*) have supported earlier conclusions with new measurements of virus obtained directly from leaf hairs of infected plants. Sigurgeirsson and Stanley (*6*) have also measured the virus particles in freshly expressed juice and found them more uniform than those in older or in centrifuged material. Later results (*3, 4*) show that the greatest number of virus particles (20–40%) fall into a group size of 15×280 mμ, and that less than 5% are extraordinarily short or long. It is emphasized by these workers that much of the variation in length is attributable to aggregative or disruptive forces occurring during preparation of the virus concentrate for examination under the electron microscope.

In connection with certain virus studies made in this laboratory, it has been found that the application of direct water pressure to plants systemically infected with mosaic yields clear exudation drops containing a sufficient virus concentration to be observed when applied to the electron microscope screens. The approximate concentration of individual droplets may be checked by wiping drops on a local lesion host with the tip of a finger or a glass spatula. This exudate contains some extraneous material; but, with the gold shadow-casting process, the virus particles were easily found in considerable numbers. In the case of plants with hydathodes, the virus may be exuded from these natural openings, but with other plants the droplets were collected chiefly from the cut ends of veins.

This method of obtaining a relatively pure form of virus was described from this laboratory in 1924 (*1*). The method has also been illustrated in other papers in connection with water-congestion studies (*2*). Where larger quantities of virus are required, as for purposes of concentration by differential centrifugation, a more recent modification has consisted of collecting the exuded drops in a 14″ metal side-arm funnel properly placed under the plant foliage. In this manner 25–50 cc may be collected in a few hours from suitable plants, especially if the leaf blades are cut so as to expose the ends of numerous veins. The virus concentration of this exudate is not usually as high as that secured by the usual procedure of expressed juice. However, practically all of the extraneous plant material that interferes with electron microscopy is eliminated. With the water-pressure method, the exuded droplets may be applied directly to the collodion film of the electron microscope screen. Drying in a desiccator, followed by gold shadow-casting, completes the shortened preparation for electron microscope examination. Several strains of the tobacco mosaic virus particles have been collected in this manner, and different host plants, especially tobacco, tomato, *Eryngium aquaticum* L., and *Digitalis lanata* L. (a previously unreported host species), have been used. The method is applicable to glaucous plants such as *E. aquaticum* and *D. lanata*, where the leaf-hair method would not be possible.

The pressure exudate method of preparation of the virus for electron microscopy should not cause aggregation or disruption of particles of the infectious unit, especially when the first drops are used, since the actual pressure at the point of exudation is very low. Variation from the normal virus length in the droplets, if such occurs, would necessarily seem to arise from the drying process on the screen. The latter supposition is discounted by those who have found the virus particle relatively uniform in length (*3*). Measurements of the virus particles obtained by the water-pressure method have not yet been made. The electron micrographs, however, show a high variability in the length of the particle, not approaching, for example, the uniformity exhibited by most cultures of microorganisms.

This method of obtaining virus may prove useful for other studies by means of electron microscopy.

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